

A MANUAL *of the* ASPERGILLI

By

CHARLES THOM

*Collaborator Northern Regional Research Laboratory
Formerly Principal Mycologist Bureau of
Plant Industry U. S. Department of
Agriculture Washington D. C.*

and

KENNETH B. RAPER

*Senior Microbiologist, Fermentation Division Northern Regional
Research Laboratory Bureau of Agricultural and
Industrial Chemistry, U. S. Department of
Agriculture Peoria, Illinois*



BALTIMORE
THE WILLIAMS & WILKINS COMPANY
1945

COPYRIGHT 1945
THE WILLIAMS & WILKINS COMPANY
Made in the United States of America

Published May 1945
Reprinted January 1951

COMPOSED AND PRINTED AT THE
WAVERLY PRESS INC
for
THE WILLIAMS & WILKINS COMPANY
BALTIMORE MD U S A

CONTENTS

PREFACE	vii
PART I GENERAL DISCUSSION	1
Chapter I Historical Introduction	3
Chapter II Classification Generic Diagnosis and Synonymy	6
Chapter III Morphology and Description	10
Chapter IV Cultivation and Examination	31
Chapter V Preservation of Cultures	50
Chapter VI Variation	63
PART II THE MANUAL PROPER	79
Chapter VII The Use of the Manual	81
Chapter VIII The <i>Aspergillus clavatus</i> Group	92
Chapter IX The <i>Aspergillus glaucus</i> Group	100
Chapter X The <i>Aspergillus fumigatus</i> Group	148
Chapter XI The <i>Aspergillus nidulans</i> Group	155
Chapter XII The <i>Aspergillus ustus</i> Group	171
Chapter XIII The <i>Aspergillus flavipes</i> Group	179
Chapter XIV The <i>Aspergillus versicolor</i> Group	183
Chapter XV The <i>Aspergillus terreus</i> Group	195
Chapter XVI The <i>Aspergillus candidus</i> Group	206
Chapter XVII The <i>Aspergillus niger</i> Group	214
Chapter XVIII The <i>Aspergillus wentii</i> Group	241
Chapter XIX The <i>Aspergillus tamarii</i> Group	250
Chapter XX The <i>Aspergillus flatus-oryzae</i> Group	259
Chapter XXI The <i>Aspergillus ochraceus</i> Group	273
PART III REFERENCE MATERIAL	287
Chapter XXII Topical Bibliography	289
Chapter XXIII General Bibliography	319
Chapter XXIV Check List of Species and Genera	331
Chapter XXV Accepted Species Varieties and Mutations	360
INDEX	363

PREFACE

Aspergillus as the name for a genus of molds dates back to Micheli (1729) but it was not until the middle of the 19th Century that the *Aspergilli* began to be recognized as active agents in many decay processes as occasional causes of human and animal disease and as fermenting agents capable of producing valuable biochemical products. Various taxonomic efforts were made. DeBary, Fresenius, van Tieghem and others described the particular species that they used. Bunier described in brief inadequate terms all the *Aspergilli* he found. Wilhelm and Wehmer reviewed the literature, described and figured the forms they knew, these were few in number but the work was done so well that it fixed group types. In 1926 Thom and Church brought all of this material together in a monograph. The increased study devoted to the *Aspergilli* in recent years shows some of their groupings to be inadequate. In addition a large amount of new material has accumulated. The *Aspergilli* have become increasingly important as responsible agents in a number of industrial fermentations. Many of them are being found capable of producing antibiotic substances and their possible use in this field will undoubtedly be exhaustively explored. For these reasons the need for a manual for those who wish to identify *Aspergilli* under observation without regard to the historical aspects of the group has become increasingly apparent.

This book is definitely a manual not a monograph. It is based upon comparative study of thousands of strains of *Aspergilli* in culture. Representative strains giving the range of morphology and biochemical activity in each species are maintained in the permanent collection of the Northern Regional Research Laboratory. Consistent efforts have been made to obtain the organisms actually used by authors who have put forward new nomenclature. The manual thus seeks to present under species names only living cultures known to the authors although it seemed advisable to make a few additions based upon literature. The species included are arranged as far as possible into natural groups which bring together aggregates of strains or species agreeing in important morphological characters. For the most part physiological or biochemical information if available indicates related activities within these groups. The names selected for use appear to be taxonomically correct. A large number of names are necessarily rejected. If known to belong to some unidentifiable member of a group or believed from literature to be correctly placed there each of these names is accounted for in the discussion of the group. If the information available does not justify allocation to some species or species

aggregate, the name will be found in the check list with any information at hand. Large gaps in our information about the *Aspergilli* still exist. Some of these are pointed out in the text. The great activity of the present day will undoubtedly render any arrangement of the *Aspergilli* obsolete sooner or later, but it is believed that the classification put forward here is at least temporarily practical.

Recognizing that any species name for an *Aspergillus* appearing at any time in the literature may at some future time become important for some unanticipated reason, an alphabetical check list giving each of the names found, the author, the date, and the place of publication has been included. An index reference to page in the manual, or the method of disposal of the name, is added to bring the material to its greatest usefulness as a ready reference.

Two types of bibliography are presented: a general bibliography, alphabetical to author's name and sub-indexed as to date of publication when necessary, includes authors of species and other investigators whose work is cited in the text. In addition a topical bibliography is presented. Although incomplete, it is hoped that the latter will assist greatly in the search for special literature on particular subjects. Believing that the more recent literature on these subjects will generally be of the greatest interest and value, the material is presented chronologically. Duplication between the two bibliographies may or may not occur.

A manual, if it is to facilitate the identification of these molds by the actual worker in the laboratory, must present descriptive and illustrative material in as simple form as seems consistent with sound scholarship. From our present point of view, the describer of a mold must know that mold in fruiting form under the microscope as known to the early mycologists, and know it also in the culture tube. It must be isolated as a pure culture and its life history and reactions followed out upon laboratory media. Its definite place in some one of the aggregate species or groups of *Aspergilli* should be thoroughly established, then, by careful study and comparison; proper nomenclature should not prove difficult.

This manual then seeks to serve two purposes: (1) to provide the worker encountering an *Aspergillus* with means for its identification, and hence to open to him the whole literature of the group as well as the particular species, and (2) by enumerating all forms found in the literature and indicating their proper allocation, to guide the user of that literature in the interpretation of names found in his reading but not known to him in nature, in culture, or in exsiccata.

The authors acknowledge the cooperation of Dr. Johanna Westerdijk, Dr. F. H. van Beyma, and their colleagues at the Centraalbureau voor Schimmelcultures, at Baarn, Holland, in the free exchange of cultures and

information. Professor Ph. Biourge and Dr. Paul Simonart at Louvain put their entire collection at our service after preparing and demonstrating their interpretations in their own laboratory. Dr. Raoul Mosseray sent his extensive series of variants in the *Aspergillus niger* group as accumulated from the Belgian Congo. Dr. Adalbert Blochwitz made many comments and criticisms in his numerous letters. Professor Harold Raistrick and Mr. George Smith submitted all strains reaching their laboratory with full notes on their own interpretations. Cultures and photographs some of which are used in this manual have been furnished by Messrs. John and Edward Yull. Series of cultures have been received from Drs. Marie B. Morrow and J. J. Taubenhaus in Texas. Drs. Roberta Ma and Y. K. Shih in China. Drs. G. Kita, R. Nakazawa, J. Hanzawa, and K. Oshima in Japan. Drs. G. R. Bisby and G. A. Ledingham in Canada, and Dr. H. Macy in Minneapolis, as well as individual strains from many correspondents. The laboratory collection owed much of its completeness to the punctilious workmanship and painstaking scholarship of Dr. Margaret B. Church.

In the preparation of the manuscript outstanding contributions have been made by Dorothy F. Alexander who prepared the line drawings and assisted generously in the checking and proofreading of the textual material by Mr. Roland W. Haines, Photographer of the Northern Regional Research Laboratory, who made all the color pictures as well as many of the black and white photographs, and by Miss Nancy Brant who typed the manuscript in its final form.

The authors are indebted to the Chas. Pfizer and Company Inc., Brooklyn, New York, for underwriting the cost of reproducing the natural color photographs.

Administratively, the preparation of this manual was made possible by the vision of Dr. O. E. May, Chief of the Bureau of Agricultural and Industrial Chemistry, Mr. H. T. Herrick, Director of the Northern Regional Research Laboratory, and Dr. Robert D. Coghill, Chief of the Fermentation Division of the Northern Regional Research Laboratory, who have developed the industrial exploration of the biochemical utilization of the fungi over many years.

THE AUTHORS

PART I

GENERAL DISCUSSION

CHAPTER I

HISTORICAL INTRODUCTION

Historically the *Aspergilli* as a part of moldiness of things have always been a factor in man's environment, but for ages were brushed away as white yellow green red or black mold with or without any attempt at interpretation. After the development of the microscope men began to see structure. Micheli (1729) distinguished conidiophores and heads. He noted that the heads were rough the spore chains or columns producing an uneven surface hence he gave the name *Aspergillus* (rough head). He then marked with Latin phrases his sketches of differently colored moldy substances for example *Aspergillus capitatus ochroleucus* probably some strain of *Aspergillus ochraceus* *Aspergillus capitato pulla* for a black form etc. Other authors followed using much the same terminology but without illustrations definite enough to give knowledge of the structure of the heads. Thus Haller in 1742 put what appears to have been *Sporodinia* into the genus as *A. ramonissimus* etc. There is just about enough certainty in the use of *A. albus* *A. niger* *A. capitato pulla* *A. purpureus* etc., to justify the continued use of the name *Aspergillus* after taking out of the aggregate the extraneous material thrown into it by the very scanty microscopic examination given by the early mycologists.

Persoon (in the 1790's) threw the *Aspergilli* into his polyglot concept *Monilia* based upon the production of spores in chains resembling strings of beads. He made no record concerning their origin. Then Link in 1809 went back to Micheli and based his rejection of *Monilia* upon the specification that the chains of spores must have their origin in a head (capitulum).

Link failed to examine that head closely enough to keep out questionable forms although we know that in describing *A. glaucus* he had under his microscope one of that group as it was found then and now upon partly dried herbarium specimens. Correct interpretation of the structure of this head appears first in the work of Corda who began about 1828 to publish his studies of fresh material as seen under his microscope. Up to about 1850 each worker was prone to look at his predecessor's descriptions and figures and either assign whatever he had to another man's species or conclude that each specimen he had was new and add another group of names. Montagne complained (1856) that none of the descriptions written before Corda were identifiable while some of us are equally uncertain of our ability to interpret Montagne.

DeBary's laboratory in the early 1850 s seems to have introduced sufficient culture of the molds found to form the beginning of a permanent literature. This started with the recognition that the yellow perithecia called *Eurotium herbariorum* by Link, which developed among the heads of *Aspergillus glaucus* upon his herbarium specimens, were actually borne upon the same mycelium (fig 7). Fresenius Cramer, Wilhelm and Brefeld in Germany followed. Raulin and van Tieghem in France developed the fermentation of the tannins in gall nuts to gallic acid in the 1860 s with comparative study of other molds as a corollary. In 1880 in Paris Bainier began publishing his studies of molds as they appeared in pharmaceutical products. He was followed by Gueguen the Sartorys, and others in France and somewhat later by Biourge in Louvain, Belgium.

Wehmer, in Hanover began publishing his biochemical studies in 1891, which led him to develop his more pretentious monograph published in 1901. Blochwitz undertook to develop his 'system' early in the new century, but the World War delayed its publication until 1929. Meanwhile Thom and Church beginning about 1910 had published *The Aspergilli* as a taxonomic monograph in 1926. Aspergilli were listed in cryptogamic floras lists manuals and special papers of many kinds over the whole period but critical discussions were few.

In somewhat over 200 years, an enormous mass of *Aspergillus* literature has accumulated. Justice to the writers at each stage in the development of our information calls for an analysis of the conditions which surrounded its development. Practically all of the early literature was microscopical the worker confined his study to specimens brought in from natural sources, each of which was often assumed to be typical of some species. Each worker used the microscope that he had at hand and the technique of study already known to him. Life histories and comparative examination of material from many sources were disregarded. Publications appeared as parts of floristic studies of particular regions as reports of organisms found in particular lesions of man or animals or as observed in special industrial connections. After DeBary's group began to study organisms in comparative culture the number of publications began to increase rapidly. By 1929-1930 Tamiya and Morita were able to cite 2 424 titles of papers which in some way concerned the Aspergilli in their published *Bibliographie von Aspergillus 1729 bis 1928*. A mathematical analysis of this literature was published by Tamiya in 1931. Referring to his table 1 71 titles appeared in the 125 years before DeBary's 1854 paper 73 appeared in the next 18 years preceding Brefeld's 1872 papers 236 in the next 19 years just preceding Wehmer's oxalic acid reports in 1891. All of this may be called the period of physiological morphology. The remaining two thousand published between 1891 and 1928 represent the

pure culture period. This may equally well be called the biochemical period.

The taxonomic part of this literature was scattered through several languages and represented many schools of nomenclatorial thought. The man who had seen only three or four *Aspergilli* found no difficulty in separating them. Each used his own descriptive terms—adequate for his purpose but useless to the next man with different species. Saccardo just published them all. Critical analyses were not available.

In *The Aspergilli* (1926) as a monograph Thom and Church sought to bring together all of this taxonomic literature as published before that date and to present a critical opinion as to the proper relationship of the species described whether retained in the genus or placed elsewhere. Some 350 names were thus accounted for but the actual number of species accepted as known in culture or probably determinable from existing literature was given as 69 (p. 252). These were more or less arbitrarily considered in 11 groups. In undertaking to account for all the described forms it was deemed advisable to include in the various groups discussed many forms whose published descriptions were inadequate for positive identification but complete enough to indicate their affinities with known sections of the genus. Citation of these species in the older literature might therefore be traced to group relationship and in that way correlated with more recent studies of the same or related organisms. In addition certain names were listed as entirely unidentifiable and certain other forms as belonging to other genera.

Various other proposals for this purpose have been made. Blochwitz in 1929 published his long-delayed *System und Phylogenie* with interpretations and proposals for grouping quite different from those of Thom and Church. Neill (1939) reduced the species recognized to the larger aggregates paying little attention to details of head and spore formation. George Smith (1938) seeking industrial utility simplified his descriptions and introduced many photomicrographs. He discarded the literature for the most part and undertook to guide the worker to the larger groups which could be located principally by color and shape of head as shown by his figures. Dodge (1935) keyed all species whose names appear in medical literature from their descriptions but without studying them in culture.

In 1939 Biourge prepared a manuscript analysis of the genus for the Third International Microbiological Congress in New York. His associate Dr. Simonart came to represent him but left because of the war. The paper was not presented but was transmitted to us because return to the author was impossible. Biourge died somewhat later. His scheme of classification prepared in his last years is not presented because it contains many things too bizarre to do justice to a man who for many years was a master workman as well as a valued friend.

CHAPTER II

CLASSIFICATION, GENERIC DIAGNOSIS, AND SYNONYMY

Class Ascomycetes
Order Plectascineae
Family Aspergillaceae
Genus *Aspergillus*

Class Fungi Imperfecti
Subclass Hyphomycetes
Order Mucedineae
Family Mucedinaceae
Subfamily Aspergillaeae
Genus *Aspergillus*

The above classification follows Engler and Prantl. Changes in the names of class order and family appear in various proposals without essential differences in placement. G. W. Martin would replace the names Plectascineae with Eurotiales, Aspergillaceae with Eurotiaceae and *Aspergillus* with *Eurotium* in the plea that the first name applied to the ascospore form determines the generic usage. Since the group has too many common characters to be split to advantage and since the non ascospore forms vastly outnumber the ascospore it is better to forget *Eurotium* along with the technicality. In this arrangement the name *Aspergillus* appears in its proper place among the ascospore fungi. It also appears among the Hyphomycetes properly keyed to facilitate the identification of organisms obviously related but which do not produce ascospores as far as known.

GENERIC DIAGNOSIS

There is progressive need for broadening the application of the name *Aspergillus* to include organisms whose structures as determined in culture by microscopic study point to membership in specific natural groups. There is need for analysis of the question whether the whole group shall be retained as *Aspergillus* or further divided into more closely related entities such as *Eurotium* of Link, *Aspergillopsis* of Spegazzini, *Diplostephanus* of Langeron, *Steriogmatocystis* of Cramer and perhaps others. There are so many arguments for keeping them in a single group that the characterization of the genus *Aspergillus* used by Thom and Church in

1926 has been emended and introduced here This is followed by brief considerations of the other more significant synonyms

Aspergillus Micheli in *Nova Plantarum Genera* p 212 Plate 91 1729
Compare Link, in *Obs* p 16 1809 Corda in *Icones Fungorum*
4 31 Tab VII fig 94 1840 and Thom and Church in
The Aspergilli ■ 4 1926

Vegetative mycelium consisting of septate branching hyphae colorless bright colored or in a few forms slowly becoming brown in localized submerged areas or producing brown crusts or sclerotia conidial apparatus developed as conidiophores and heads from specialized enlarged thick walled hyphal cells (the foot-cells) producing conidiophores (stalks) as branches approximately perpendicular to the long axis of the foot-cell and usually to the surface of the substrata in or upon which they are borne conidiophores unseptate or septate usually enlarging upward and broadening into elliptical hemispherical or globose fertile vesicles bearing fertile cells or sterigmata either parallel and clustered in terminal groups or radiating from the entire surface sterigmata either in one series only or as a primary series each bearing a cluster of two to several secondary sterigmata at the apex conidia varying greatly in color size shape and markings successively cut off from the tips of the sterigmata by crosswalls (not produced by budding) and forming unbranched chains arranged into radiate (globose) heads or packed into columnar masses perithecia found in certain groups only unknown in most species cleistocarpic thin walled producing asci and ascospores within a few weeks sclerotia regularly found in some strains occasionally found in other strains and not found in other and closely related strains mostly globose or subglobose composed of polyhedral thick walled cells

Eurotium Link in *Obs* p 31 Taf 2 fig 44 1809

Synonym *Mucor herbariorum* Wiggers in *Primitiae Florae Holisticae*
as No 1158 1780 See also DeBary in *Bot Ztg* 12 425
1854

The yellow perithecia suspended in networks of hyphae above or at the surface of his badly dried herbarium specimens were taken by Wiggers (1780) as the basis of *Mucor herbariorum* Link (1809) recognized the bodies as ascospore hence segregated them under the generic name *Eurotium* Then in 1854 DeBary published proof that these perithecia were borne upon the same mycelium as the actual *A. glaucus* fruits among which they developed He then called each of his *Aspergilli* *Eurotium* *Aspergillus* followed by the specific name whether ascospore strains were known or not In spite of technicalities invoked by some to bolster the

use of the name *Eurotium* for all *Aspergilli*, or failing in that for all ascospore strains most workers have accepted the numerical predominance of the non ascospore strains as ample reason for the general use of the name *Aspergillus*. For practical purposes *Eurotium* is not used here.

Sterigmatocystis Cramer, in Vrtljschr Naturf Gesell Zurich Jahrg 4, Heft 4 p 325 Taf II, figs 1-15 1859

Cramer in 1859, published his study of a black *Aspergillus* from the human ear. Since the fruiting head differed from that of Fresenius' *A. fumigatus* by showing a primary series of sterigmatic cells radiating from the vesicle, each bearing a crown of several sterigmata which in turn each bore a chain of spores he made this character the basis of his new genus *Sterigmatocystis*. Cramer's name has been accepted by many workers, but was rejected by Wehmer, Thom, and others on the proof that such use would separate strains obviously related in such a group as *Aspergillus flavus* and its allies and even among the black *Aspergilli* studied by Cramer himself. The additional name serves no useful purpose as an aid to identification, hence is not recognized here.

Euaspergillus Ludwig, in Lehrbuch des niederen Kryptogamen p 258 1892

The proposal to apply a separate generic designation to all *Aspergilli* producing sclerotia would take out the groups typified by *A. candidus*, *A. niger*, *A. wentii*, *A. tamaris*, *A. flavus* and *A. ochraceus*. No one has followed Ludwig.

Aspergillopsis Spegazzini in An Mus Nat Buenos Aires Ser 3 13 434 1911

The black spored *Aspergilli* were described as dematiaceous hence separated from all the other groups. No practical reason for accepting this proposal has been offered.

Diplostephanus Langeron in Compt Rend Soc Biol Paris 87 343-345 1922

Under this proposal ascospore *Aspergilli* with the double series of sterigmata would be separated with *A. nidulans* Eidam as type. No technical application of nomenclatorial rules justifies the complications introduced.

In addition to the above names proposed to cover blocks of species with particular characters in common, a series of names have been used

by various authors for individual species *Alliostora* for a black form *Ascothoria nigrans* for *A. niger* *Aspergillopsis* Sopp for an unidentified organism *Cladosorium* for Vuille's mutant of *A. niger* *Dimargaris* for some white forms *Emericella* and *Inengaea* for *A. varicolor* *Mucor* as a place to assign *A. herbariorum* *Sartorya* for a possible ascosporic *A. fumigatus*. These names are cited in the check list but contribute nothing to this study of the group as a whole.

CHAPTER III

MORPHOLOGY AND DESCRIPTION

INTERPRETATION OF PUBLISHED DESCRIPTIONS

Basic Assumptions In interpreting the descriptions of *Aspergilli* in a literature covering a long period of time, certain assumptions although not always justified form a working hypothesis for presumptive identification. Conidiophore walls and conidial walls are assumed to be colorless and smooth unless color or markings are either figured or described. Perithecia and sclerotia are assumed to be lacking unless the presence of such structures is specifically noted. Colors are assumed to apply to the general color scheme of the colony unless specifically applied to the conidia, ascospores or other details by the describer. Whereas colony coloration may arise from an admixture of conidial structures and varying amounts of vegetative hyphae colored or uncolored together with perithecia or sclerotia in greater or lesser numbers it is assumed to result from the massing of conidial heads unless otherwise stated.

Difficulties encountered in interpreting descriptions based upon color are less for the worker with a growing culture before him than for the one handling descriptive literature alone, since the presence of white, green, yellow, green, brown or black heads is readily distinguished with a hand lens even though sparingly produced upon a colony in which another color predominates as in many members of the *A. glaucus* group or in *A. flavipes*. The color of the conidial heads is often made the primary basis of species description.

Extent of Study Interpretation of descriptive literature accompanied and supplemented rather than preceded the study of great numbers of cultures so that the groups established are based upon the actual handling of thousands of cultures representing hundreds of forms of *Aspergillus* handled during a period of more than thirty years. Many of these were studied on natural substrata before their isolation. In addition examination of exsiccata from several large herbaria while more or less unsatisfactory as to detail in identification of species furnish confirmatory evidence of the soundness of the groupings proposed.

Types For a few of the specific names in use today the type strain has been definitely maintained in culture. For most series selection of a morphological entity to give a concrete concept back of the use of a name becomes a matter of critical judgment. For the purposes of this manual an attempt has been made to base the use of the individual name upon the

morphological picture most frequently encountered rather than upon a selected strain assumed to be but not known to be the one first described.

Descriptive terms. For purposes of description a standardized use of terms has been adopted. Great diversity is encountered in the literature in various languages. Even translated into Latin Saccardo never homogenized the terms so that succeeding descriptions upon the same page use descriptive terms in the same sense. To make comparison with existing literature more convenient the usages defined in the following pages will cover the morphology as definitely as possible and indicate the usages found in the older literature. To serve as a basis for the collection, interpretation and presentation of pertinent information regarding *Aspergilli* to be studied a guide sheet of the type used by the authors is presented in the introductory portion of the manual proper (p. 82).

THE *ASPERGILLUS* COLONY

Since few of the *Aspergilli* regularly produce perithecia and ascospores a basis for identifying the majority of the molds of this group as they are actually encountered in nature and in culture must be found in the description of the colonies and in the details of morphology found in the spore-bearing structures available.

The vegetative mass of most *Aspergilli* consists of submerged mycelium from which only fruiting hyphae rise above the surface. Such colonies suggest a field of ripening grain in which conidiophores and ripening heads predominate and have been described as velvety (the German term used is *raue*) from their appearance in many species. Some species produce a more or less aerial felt (floccosity) of branching and interlacing hyphae bearing conidiophores. This is characteristic of certain strains of the *A. terreicolor* and *A. fumigatus* groups upon Czapek's solution agar and other culture media commonly employed and it is normally one of the most striking characters of *A. wentii* under laboratory cultivation (fig. 1 B). Many strains of the *A. glaucus* group form long streamers of hyphae hanging from meat stored in cool damp rooms and certain of these retain this character in laboratory culture. The character of the surface growth is a diagnostic characteristic which is usually fairly reliable under reasonably uniform conditions of culture.

In describing the *Aspergillus* colony cognizance should be taken of such factors as age, rate of growth, temperature of incubation and the composition of the substratum. Provided with this information subsequent investigators can intelligently interpret their cultures in terms of species previously described.

Many species and strains of *Aspergillus* often produce conspicuously zonate colonies. Most commonly these take the form of fairly regular

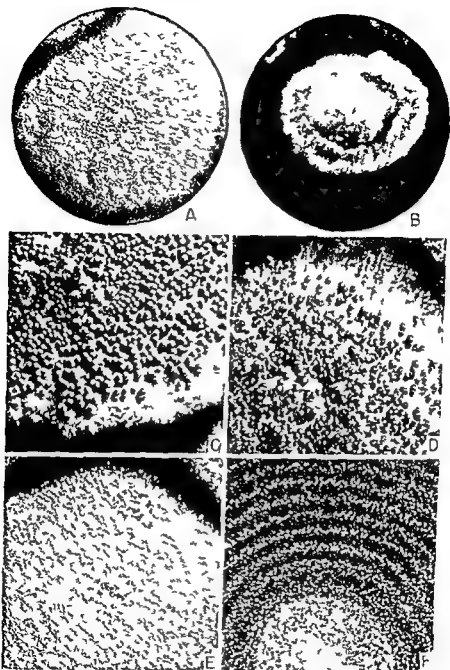


FIG 1 Colony types in the Aspergilli. A *Aspergillus parasiticus* NRRL No 465 heavy sporing non floccose colony on Czapek's solution agar room temperature 10 days $\times 1$ B *Aspergillus wentii* NRRL No 375 light sporing floccose colony grown under the same conditions $\times 2$ C *Aspergillus varicolor* NRRL No 1954 characterized by the production of abundant large perithecia $\times 3$ D *Aspergillus alia eus* NRRL No 315 characterized by abundant black sclerotia $\times 2$ E *Aspergillus ochraceus* NRRL No 408 on Czapek's solution agar showing heavy sporing essentially azonate colony $\times 1$ F The same on hay infusion agar strongly zonate $\times 2$

as in *A. niger*, or the heads may be uncolored or nearly so, while the outer layers of the conidiophore wall may be colored as in *A. flavipes* and *A. ochraceus*. In some species of the *A. glaucus* group, the colony color is at first green with the development of conidia, then predominantly yellow to ferrugineous from ripening perithecia. Again, in other species of the same group the walls of the conidiophores and, more particularly, aerial hyphae become encrusted with granules that are characteristically yellow in the young colony, but become reddish or ferrugineous in age. Such colonies are at first predominantly yellow with green heads inconspicuous on a yellow background and later become rusty red or brown.

In the *A. flavus* group, Saito (1907) followed by Thom and Church (1921, p. 115) found that cultures with brighter shades of green when subjected to a vapor of ammonia would lose the green color and assume the somber yellow shades of variant members of the same group and that this reaction was reversible, since the vapor of acetic acid would restore or even intensify an original green shade. The experiments pointed to the hypothesis that the wide range of shades produced by mixtures of yellow and green in the *A. flavus* or *ae* group may be attributed to racial limitations strain by strain, in the range of hydrogen ion concentration produced by metabolism. When a carbohydrate fermentable by the particular species is present an acid reaction is promptly produced in the growing colony, as growth progresses alkaline products are also produced. The colony color in this series, therefore, reflects first the intensity of the initial acidity as shown by the intensity of the green color reached. The persistence of this shade or its subsequent reduction or entire disappearance to leave a somber yellow or finally brown colony represents the balancing of the two activities. As a result, certain strains of this group if grown on Czapek's solution agar, are quickly and very persistently deep green others become particular shades of green which fade to yellow and finally some of them to brown and a few forms produce no true green color but assume a somber yellow with the first development of conidia.

Color changes in the conidia have not been satisfactorily worked out. In the *A. niger* group the shade of yellow to purple-brown or black seems to be a strain or race character little influenced by handling which is not destructive of the racial entity. Each race seems to reach a fixed quantitative limit in the secretion of the coloring substance thus reducing the most conspicuous diagnostic character among closely related forms to a quantitative rather than a qualitative basis of separation.

Color in Conidial Walls

The conidial walls may be smooth but carry sufficient coloring matter in diffused form to give the characteristic colony color. Within such

series as *A. fumigatus*, *A. nidulans* and *A. ochraceus* however strains or races may be found which vary from conidial walls smooth or nearly so to walls bearing echinulations or even traceries apparently produced by aggregation of color substance into spinules or bars between the outer and inner walls of the cells. Although smoothness and echinulation have received much weight in descriptive literature observations such as the above would indicate that this character should only be used to separate nearly related strains in the same group rather than as a group character.

Literature on the nature of color in *A. pergilli* seems to begin with Iino's study of aspergilline as produced by *A. niger* in 1891 this was followed more recently by Quileco and Quileco and Di Capua in 1933. Disregarding the record of observations only more pretentious work appeared when Bainier and Sartory undertook to use color production to separate members of the *A. glaucus* group about 1910 to 1912. Their experiments supplemented observations of colonies checked against a color chart with a routine series of solubility and precipitation tests. Blochwitz (1929-1935) followed by using a series of routine solubility tests against all species producing bright colors but failed to coordinate his tests to show the relation of test to culture medium and conditions and to age of the culture studied. Later Gould and Raistrick (1934) and Raistrick, Robinson and Todd (1937) studying the *A. glaucus* group extracted and defined the colors found but again failed to follow the transformations in the color of the particular species during the course of colony development. Until someone correlates color determination and composition more closely color observations will continue to be useful accessory data which must be related to the age of the colony and to the composition of the medium as closely as possible to have value.

Colors in the Substratum

Production of bright colors in the substratum is frequent among the *Aspergilli*. The color produced by any species or race in any medium is dependent first on the ability of the mold to elaborate the particular product and second on the presence of the necessary building material in the substratum. A mold may grow well upon a particular medium without discoloring it; a transfer from this colony to another substratum may turn the second medium red or yellow.

Color in the substratum is the result of the particular *Aspergillus* acting upon the particular medium under a certain range of temperature. Most of the species of *Aspergillus* if they produce any color produce from a trace to abundant yellow in the early stages. This may persist or give place to shades of orange red or purple. In some cases the color fades out as the colony becomes older. In descriptive work progressive changes

in intensity of color, or the presence or absence of color in different substrata, make the use of closely defined shades or intensities of color in the substratum an unreliable means of characterizing cultures

Observations of colors produced in the substratum remain, however, very conspicuous and exceedingly useful accessory characters which aid in the placing of species. The describer must bear in mind that such color reactions are confirmative not absolute characters in separating species.

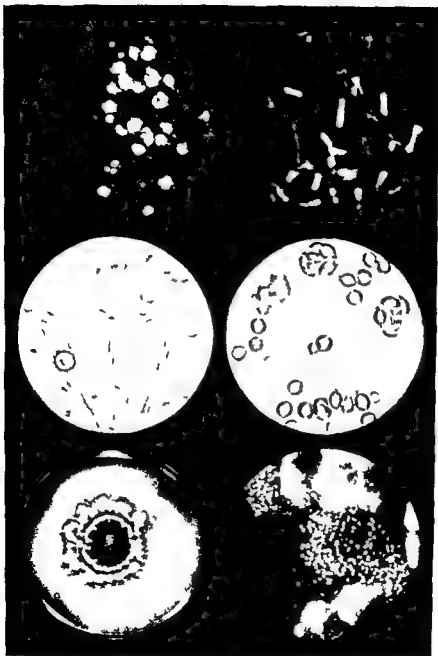
The final difficulty in dealing with color as a separating character rests in the loose use of color names, which is only partially corrected by the use of color standards. Comparison of the same culture by different individuals introduces very considerable discrepancies which become serious when a specific descriptive name or number from one of these standards is introduced into a technical description. In general, a series of observations giving a range of colors for a species is less liable to introduce errors of subsequent identification.

MORPHOLOGY

From the time of Micheli, the name *Aspergillus* (literally, rough head) has been used for molds with a conidiophore or stalk and spore bearing head (capitulum).

The Head

The first structure observed in a detailed study of the colony is the spore-bearing head. The color, shape, size and arrangement of such heads are characteristic of the species and to a lesser extent of the groups to which they belong (figs 4 and 5). Wehmer (1901) roughly grouped his *Aspergilli* into *Microaspergilli* and *Macroaspergilli* on the basis of the size of the fruiting parts. Thus *A. fumigatus*, *A. nidulans*, *A. sydowii*, and *A. versicolor* would represent *Microaspergilli* while *A. niger*, *A. clavatus*, *A. ochraceus*, *A. wentii* and *A. tamaris* would be readily classed as *Macroaspergilli*. The distinction breaks down when great numbers of forms are studied, but the comparative size of heads and conidiophores remains a useful adjunct in description. The heads in certain species show a consistent range of measurements and form, as in *A. fumigatus* and *A. nidulans* which have heads of small diameter forming columnar masses (fig 4). Similarly characteristic heads of *A. niger*, *A. ochraceus*, or *A. wentii* are globose and large (fig 5). In other species notably in *A. flavus* or *A. candidus* (fig 60) several sizes and shapes of heads are regularly found in the same colony. The range of size and shape, however, remains characteristic. The observation of many heads in the colony and preferably in many separate cultures forms a better basis for description of sizes and measurements than limited observation. Further description



Pl. I

A D 4 pe gtl dnl (Td) W t NRRI N 191 4 (1 pe lft port (col) 1
 d l i g pe tler d b d ex l l head B (1 pe right co dual leads t g t i col col
 d col mnar t X 60 (for t lft photo en graph for d l res l t w g brow w l d co l
 phore d gve n l m X 60 D ee to all conpore rel val t h l w t n g w l n l re
 l are (rol X 100 E 1 F A p g B / R pe 1 TI m NRRI N 18 E d l l ft
 gl col y l row l l t talk l g e r / j ce tral res l l talk l t t de l loc l
 used g al res th y n w i te flocone to be a co pased l ly f h H cell f t l right
 port on (sam colo) son h t nla ged X 8 (C l e phot graph by H nee N rth R g: nal Rese reh
 Laboratory R prod ced thro gh co-operatio (Ch PE & Co l)

of the structure of the head is given in the more logical order, following the discussion of the stalk or conidiophore

The Foot Cell

The first step toward conidium formation in the *Aspergilli* is the differentiation of certain cells (the foot-cells) in the mycelium for propagative purposes. These cells become larger, thick walled, and each usually bears a single conidiophore as a branch perpendicular to the long axis of the cell (fig. 2 A) and usually about midway between the ends of the cell. In age these cells frequently become fantastically curved and twisted with their connection to vegetative hyphae inconspicuous but usually still determinable. These foot-cells are commonly submerged in the substratum although there are a number of strains of the *A. glaucus*, *A. fumigatus*, *A. terreus*, and especially of the *A. flavus-oryzae* groups in which the conidiophores arise in this way from arial hyphae. In *A. effusus* the foot-cells are frequently long and several of them connected together to form whole hyphae bearing considerable numbers of very short conidiophores (fig. 71 B₂), hence their differentiation from the sterile or vegetative cells is less easily determined. Failure to recognize the foot-cells as present in the *Aspergilli* led Ferdinandsen and Winge (1920) to describe *S. dipus* using the foot-cell as the principal diagnostic character of the species. The presence of such a differentiated foot-cell is proposed as an arbitrary character to be used in separating certain depauperate forms of *Aspergilli* which approach the structure and appearance of the monoverticillate *Penicillia* (*Citromyces*) from the *Penicillia*. Organisms which lack the typical *Aspergillus* head with its conidiophore and especially its foot-cell may be best classified elsewhere.

The Conidiophore or Stalk

The erect perpendicular branch from the foot-cell constituting the conidiophore usually enlarges upwards toward the apex at which it dilates more or less definitely to form the vesicle (fig. 2 C-E). The section of the conidiophores from the foot-cell to the base of the conidial head is measured and reported in describing species.

The conidiophore in some groups is not only septate but each cell is sufficiently distinct to justify the term *articulate* which is frequently encountered in the older descriptions. In our experience in examining specimens, articulate conidiophores are found only in the *A. glaucus* group. In most *Aspergilli* the unity of the whole conidiophore is fairly accentuated. Septa if present are thin, fragile, and inconspicuous; the whole conidiophore is enclosed by continuous characteristically thickened walls without conspicuous nodes as an evidence of septation.

Thickening of the conidiophore wall may be uniform or may be greater at the base, thinning to negligible toward the apex. Two general sections

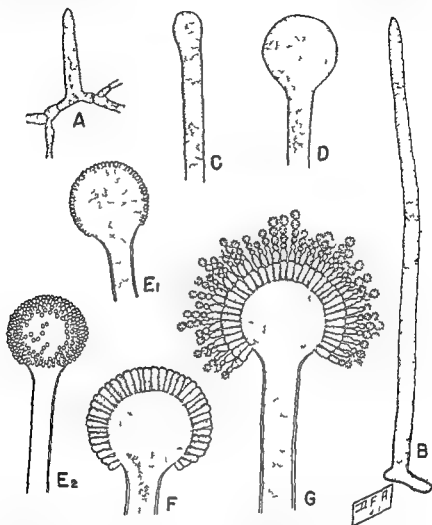


FIG. 2 Development of the conidial apparatus in *Aspergillus niger*. A Foot cell bearing young conidiophore as a vertical branch. B Developing conidiophore. $\times 172$. C and D Development of the vesicle by swelling of the terminal portion of the conidiophore. $\times 265$. E₁ and F Vesicle in optical section and surface view showing early development of primary sterigmata. $\times 265$. F Later stage in development of primary sterigmata. $\times 265$. G Young fruiting head showing secondary sterigmata bearing chains of conidia. $\times 265$.

based upon the character of the thickening of the conidiophore wall are fairly readily distinguished although the careful use of high magnification is occasionally necessary to separate certain strains. In the first the

outer surface of the wall is free from pits warts or roughenings hence is called smooth its structure is difficult to differentiate the mass of the cell wall appears homogeneous or nearly so under the microscope and does not absorb the ordinary protoplasmic stains In some species the inside

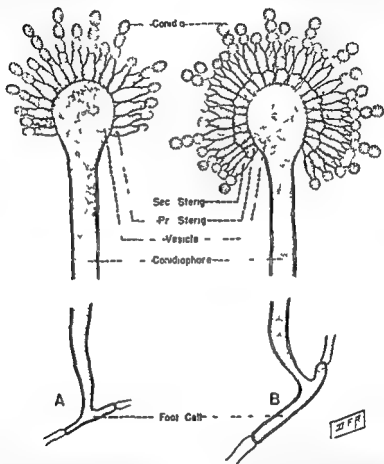


FIG 3 A *Aspergillus niger glaucus* NRRL No 127 typical head showing only one series of sterigmata $\times 575$ B *A. versicolor* NRRL No 239 typical head showing sterigmata in two series $\times 1700$

surface of the wall shows irregular clumps or uneven thickenings When broken many of these conidiophores show uneven or jagged ends like a broken glass tube in the *A. niger* group the broken ends split like bundles of laths (fig 64 B) giving a possible clue to the method of their formation In the second section the wall appears dotted or pitted or as interpreted



FIG. 4. Conidial heads group types. A *Aspergillus clavatus* group conidial heads of *A. giganteus* NRRL No. 119 showing typical clavate form, $\times 15$. B *Aspergillus glaucus* group heads of *A. niger glaucus* NRRL No. 127 showing characteristic radiate pattern $\times 35$. C *Aspergillus nidulans* group heads of *A. nidulans* showing typical short columnar form $\times 35$ (Photograph by Edward Yuill). D *Aspergillus flavipes* group heads of *A. flavipes* NRRL No. 1959 typically barrel form or loose columnar as shown $\times 11$. E *Aspergillus terreus* group. A *terreus* NRRL No. 265 heads columnar of uniform diameter throughout often becoming quite long as shown $\times 22$. F *Aspergillus ustus* group. A *ustus* NRRL No. 1974 heads typically loose and radiate as shown under certain conditions approaching columnar $\times 22$.

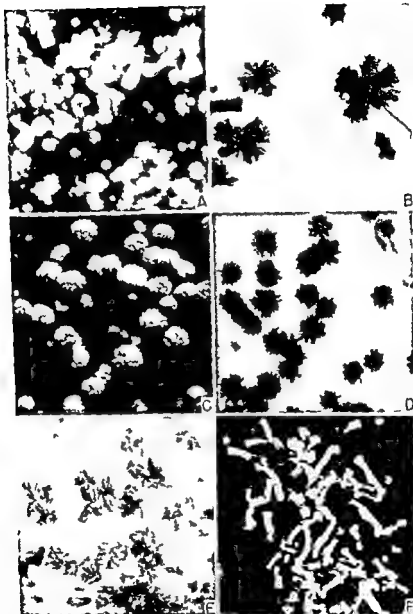


FIG 5 Conidial heads group types A *Aspergillus candidus* group *candidus* NRRL No 308 showing characteristic mature heads of different dimensions $\times 18$ B and C *Aspergillus niger* group B strain NRRL No 67 showing typical mature heads $\times 30$ C A *niger* mutant *schiemanni* heads typically globose shown (Photograph by Edward Yuill) $\times 30$ D *Aspergillus wentii* group typical globose heads of *A. wentii* NRRL No 397 $\times 18$ E *Aspergillus flavus-oryzae* group A *flavus* NRRL No 1957 typical mature heads $\times 18$ F *Aspergillus ochraceus* group A *ochraceus* NRRL No 398 typical mature heads splitting into divergent columns of conidia $\times 18$

with low magnification often rough or echinulate (e.g. *A. flavus* or *A. glaucus* group). The secondary thickenings in such conidiophores appear to have been laid down around protoplasmic areas, which for a time at least maintain contact with the primary wall outside. The size and abundance of the pits in the mature conidiophore wall differ with the species, but in a general way correspond with the rate of withdrawal of the protoplasmic mass from its primitive connection with the original outer wall. In some of the species in both groups warts, or superficial and usually more or less hemispherical concretions are found on the outer surfaces of the conidiophore wall, sometimes few and scattered widely, again fairly numerous but always unevenly distributed (e.g. *A. ochraceus* group). These warts or concretions appear to be deposits of excreted substance possibly due to the evaporation of the numerous drops or globules of liquid abundantly visible upon the young and growing conidiophores.

The color of the conidiophore wall may be homogeneous or the layers may differ markedly in shade. In a number of the groups the entire wall is hyaline. In certain other groups the outer layer is yellow as in *A. ochraceus* and *A. flavus*, or it may be some shade of green, brown, or avellaneous. In some species the whole wall is colored for all or part of its length. No explanation of these color differences is available, except possibly the varying concentration of aspergilline (Linosier, 1891) in the upper part of the conidiophores of members of the *A. niger* group as well as in the conidial heads.

The Vesicle

The conidiophore is usually much larger toward the apex than at the point of origin. At the base of the head a further dilation occurs more or less abruptly to produce the vesicle (*blase* of the German mycologists). This vesicle is globose, hemispherical, elliptical or long clavate in various groups of the Aspergilli and furnishes an enlarged surface for the attachment of spore bearing cells. The lumen of the vesicle is continuous with that of the upper part of the conidiophore; a septum near the base of the head is occasionally but only rarely seen (see Corda *A. mucronoides* for description) and has not been regularly found in any species.

Sterigmata (Compare Fig. 3)

The conidia bearing surface represented by the fertile area of the vesicle is closely covered by the simultaneous development of a layer of cells, the sterigmata, each in a general way perpendicular to a point on the fertile surface of the vesicle. In figure 3A a single layer of such cells is shown, each of which produces an unbranched chain of conidia. In figure 3B each of the first series of cells or primary sterigmata bears two to several

cells the secondary sterigmata forming a crown or verticil at the apex. Each of the secondary sterigmata bears one chain of conidia. The mechanism shown in figure 3B would produce several times as many chains of conidia as figure 3A. Such a head as figure 3B would be compact whereas figure 3A would represent a loose head.

In figure 3A the cells in the single layer each producing a chain of spores are in the strict sense the sterigmata. In figure 3B cells of the first layer or primary sterigmata produce verticils of cells the secondary sterigmata each of which is in the strict sense a sterigma. The cells are usually characteristic in size and shape for series of closely related species. Where there are both primary and secondary series the primary sterigmata are essentially supporting cells and vary much more in size and shape than do the secondary sterigmata. For this reason they are more useful in species diagnosis than the secondary series.

Various usages are found in the literature. The primary sterigmata are often called *basidia*. The secondary sterigmata are called *phialids* because they have somewhat the shape of the pharmacist's phial (vial). In translating descriptions into the Latin Saccardo apparently followed the describers verbatim hence used no consistent terminology. We find the primary sterigmata as sterigmata basidia or pseudobasidia and the secondary series as sterigmata pseudosterigmata ramuli (ramulis *sporiferis*) or even rami (branches) all of these names have been homogenized here into primary and secondary sterigmata.

Conidium Formation

The actual spore-producing cell or sterigma is definitely specialized. It ordinarily consists of an essentially cylindrical body which after reaching a length more or less uniform for the species narrows into a spore-producing tube whose diameter is fairly uniform within the species. Elongation is thenceforth confined to this spore-producing tube. The nuclei in the sterigmata divide and one of each pair of daughter nuclei passes into the tube cell division follows. Parallel with the repeated division of the sterigma nucleus the tube continues to elongate rapidly successively cutting off new sections and pushing the older cells outward. Each such chain of spores typically consists then of series of equal sections cut from one tube or tip of a sterigma and each carries a daughter nucleus derived directly from the active nucleus of the sterigmatic cell at the base of the chain. No further divisions occur among the cells in the chains. Such chains often contain several hundreds of spores or conidia each of which is theoretically at least exactly like the rest hence fully capable of propagating the species (fig. 6).

The Conidium, or Spore

The conidia are thus specialized propagative cells asexual in origin, produced by a complex cellular fruiting structure. This consists (1) of a foot cell connected with the vegetative mycelium and usually imbedded in the moist substratum (2) of a conidiophore or stalk rising more or less vertically into the air to a distance typical of the species and enlarged at the apex to form the vesicle which is the central unit and (3) of a dilated head consisting of one or two series of cells, the outermost of which are specialized for the purpose of producing chains of cells (the conidia) each equally capable of carrying the genetic factors necessary to propagate the species.

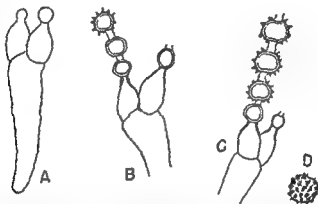


FIG 6 Camera lucida sketches showing progressive stages in conidium formation, $\times 700$. A Initiation of conidium formation. B Secondary sterigma bearing a chain of three conidia the outermost developing characteristic roughenings by the deposition of coloring matter between the outer (thin) and inner (firm) wall. C Sterigma bearing a chain of conidia in which differentiation of the outermost spore is complete. D A single mature conidium seen in surface view. See discussion on page 24.

After the conidium is separated by a septum from the mother cell or sterigma it remains so attached¹ as to draw nutrients from the parent cell at first while it assumes the size and shape characteristic of the species then it lays down within its original or primary wall a secondary cell wall whose color, texture and marking are those of the species. The secondary wall completely separates this spore from the parent cell (fig 6). Exact uniformity is not attained. An occasional cell fails to develop some differences in size are usually evident markings while characteristic in nature and general pattern, are not always identical as to details. For descriptive purposes size ranges are therefore more important than exact measurements and the nature of the markings found are more important than the relative

¹ Buller (Researches on Fungi V Chapter II 1933) discusses the primary septum as having a central pore through which connections are maintained.

number and dimensions of such. Such conidia may be very thin walled delicate and readily destroyed or firm walled almost impervious to stains and able to retain their vitality for many years. They are extremely small light and float readily in air currents. In many species the outer layer of the spore wall absorbs water slowly hence such spores tend to float in currents of fluid or to develop as mycelia covering the surfaces of liquid media. Molds being typically aerobic normal colonies develop only on the surface of the substratum where oxygen is abundant and their spores can be discharged directly into the air. Spores developing under submerged conditions in the absence of adequate oxygen produce fragmentary and defective mycelia only.

The Connective

Descriptive literature often cites the presence of a connective or disjunctor, a bridge between conidia in the chain. This is sometimes present again absent in the same microscopic preparation and when seen it appears as a short space between spores bridged by transparent cell walls. This is exactly what it is. Cells cut off from a cylindrical tube may swell and assume subglobose form without breaking their area of contact or in the swelling and rounding up process they may partially or completely break that contact leaving the original cell wall of the tube, within which they developed as a bridge across the open space (fig. 64 C). The critical examination of the developing cells in thousands of preparations have failed to justify interpretations which assume the degeneration of every alternate cell or fantastic fusions in the production of conidia. The observation of connectives is therefore ordinarily worthless because morphologically it means nothing and it is not justified by successive studies of the same species.

Endogenous Conidia

A spore is described as endogenous if it is formed within a tube or cell wall of a previously existing cell. It may be extruded through a tube. That tube may be used once only or many times. The critical factor is the formation of a cell or spore within an existing specialized spore-bearing organ and its extrusion from that body through a fixed tube. In *Aspergillus* the tip or tube of the sterigma elongates a cylindrical section is cut off carrying the tube wall and the septum at each end as the primary wall of the spore itself. Within that primary wall the spore as an entity rounds itself up to characteristic form deposits or lays down its own wall with whatever coloration or markings may be typical of the species. The primary wall may remain separate and distinct and in the ripe spore be visible under the microscope it may be blended with the secondary wall,

or it may not be determinable on the ripe conidium by ordinary examination. In the sense of the definition above, no endogenous conidia appear in *Aspergillus*. In cases the primary walls are seen to break away if ripe spores are mounted in fluid often carrying with them the granular materials which impart the characteristic marking to the spore hence leaving the wall of an *A. niger* spore smooth (*A. luteo-niger* Lutz)

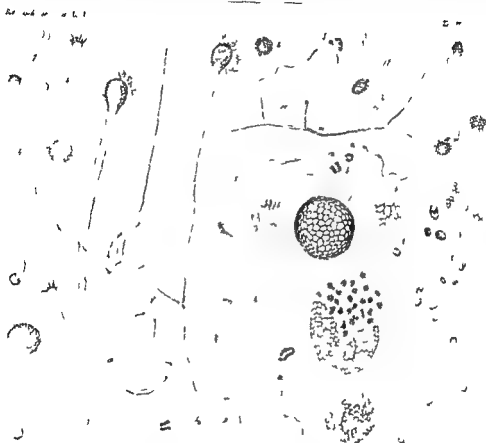


FIG 7 The development and relationship of *Aspergillus glaucus* and *Eurotium* after DeBary 1854

Perithecia

The general morphology of the perithecium of the *A. glaucus* group (*Eurotium*) was described and figured by DeBary (1854-1870) while that of *A. nidulans* was described by Eidam soon thereafter (1883). DeBary described the yellow to orange or ferrugineous perithecia as from 90 to 300 μ in diameter without ostiole and without specialized appendages (fig 7). These perithecia are borne above the surface of the substratum

and are never more than loosely hung in networks of hyphae they are often very abundant and dominate the color of the colony. Perithecium formation is favored by abundance of assimilable carbohydrate but may or may not be completely suppressed by its replacement with nitrogenous products. transfers from the same culture have developed colonies yellow from perithecia when grown on sugar solution or dense green without sign of perithecia upon a peptone medium or a piece of leather.

The perithecium of *Aspergillus* arises from a branch which coils in various manners in the different species to become the ascogone as figured by Dangeard from forms reported as *F. herbariorum*, *A. flavus*, *A. fumigatus*, *S. ochracea*, *S. nidulans* by Fraser and Chambers for *A. herbariorum* and by Dale (1909) for *A. repens*. No fertilization process was demonstrated in those studies. Dangeard (1907) reported the cells in the fruiting apparatus as multinucleate in *F. herbariorum* but to be mononucleate in the other species figured. In general however the development of an ascogone followed by the development of the perithecial wall and accessory cell masses from vegetative hyphae below the ascogone has been roughly described and figured for the group represented by *A. glaucus* and *A. nidulans* but interpretation of the other specific names used by Dangeard is doubtful even for grouping on the basis of the descriptions and figures given.

Henrard (1934) working with 15 species of *Aspergilli* reports that all of them are sexually homothallic. He summarized the literature by saying that homothallism in the *Aspergilli* has been affirmed by Kniep (1928) for *A. repens* by Schwartz (1928) for *A. ruber*, *A. repens* four other strains of *A. glaucus* and four strains of *A. nidulans* by Blochwitz (1932) for six strains in the *A. glaucus* group and by Greene (1933) for *A. fischeri*.

So far then as present information goes heterothallism cannot be assumed to account for the great variability of the *Aspergilli*.

Perithecia are regularly found in most of the species of the *A. glaucus* group and in *A. fischeri* which is closely related to *A. fumigatus* and in a series of closely related forms in the *A. nidulans* group. They are not sporadic or dependent upon unknown conditions but are regularly produced in media which are adequately supplied with sugars and the salts in routine use. Their presence in *A. wentii* and *A. citrisporus* was asserted by Thaxter (personal communication) without producing either material or description of the ascospores. Dangeard (1907) claimed to have found ascospores within the sclerotia of *A. niger* but failed to describe them. Diligent search over many years has failed thus far to confirm either statement. Still it may be assumed that such sclerotia may be the homologue of structures which under some conditions might become perithecia.

The Ascospore

So far as fully described, the ascospore of *Aspergillus* follows the general type shown in the figures and description of DeBary. In the course of its development the secondary thickening of the cell wall develops in the form of two symmetrical valves suggesting the arrangement found in the shell of a bivalve mollusk, such as the hard clam (*Venus mercenaria*). The ripe ascospore is commonly shaped as a double convex lens with the valves more or less closely in contact at the edges. A series of variations upon this basic pattern occur and characterize particular species (figs 27, 34, and 43). If the exospore is smooth and the margin of the valves is not marked by folds or ridges figure 27A characteristic of *A. repens* appears; if the exospore is rough in the absence of marginal folds figure 27D characteristic of *A. amstelodami* results; if the exospore is rough and the margin of the valves bear folds or ridges figure 43C characteristic of *A. rugulosus* and *A. fischeri* is produced. An extreme development of the marginal folds is seen in *A. varicolor* figure 43D.

When such an ascospore germinates (fig 8A), the figure first shown by DeBary (1854) develops. Exactly the same type of germination is shown by *A. nidulans* in which as the spore swells the valves first separate at one edge, then parting completely, remain on opposite sides of the germinated spore conspicuously identifiable by the bright purple red color of the valves (Thom and Church, 1926).

Hulle Cells

Mature perithecia were described for *A. nidulans* by Eidam (1883) but without giving attention to their origin. In his description Eidam pictured a loose network of hyphae more or less completely surrounding the perithecium containing large numbers of Hulle cells terminal or intercalary cells which swell and become vesiculose elliptical or almost globose then develop very heavy thick walls almost obliterating the cell lumen (fig 49). These cells were later noted by Dangeard (1907) who designated them as chlamydospores but failed to present evidence of function as propagative cells. These cells are abundant in connection with perithecia in all strains of the *A. nidulans* group but are lacking in *A. unguis* which does not produce perithecia. Cells of the same general character appear in sterile masses of hyphae in strains of the *A. ustus* and *A. flavipes* groups (fig 49) and in some strains of the *A. varicolor* group while thick walled septate hyphae at least suggestive of hulle cells appear in *Aspergillus carneus* in the *A. terreus* group (fig 49F).

In the *A. nidulans* group hulle cells Eidamsche blasen or chlamydospore (terms found in the literature) are always produced in connection with perithecium formation (fig 42). In the *A. flavipes* *A. ustus* and *A.*

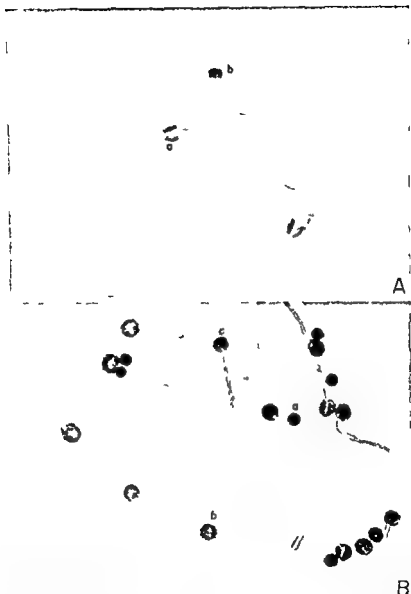


FIG 8 Spore germination. A Germinated ascospores of *Aspergillus nidulans*, $\times 750$ a ascospore in profile showing how the two valves comprising the spore wall are pushed apart as the spore content enlarges and the sporeling develops b the same in face view. B Germinating conidia of *Aspergillus niger* $\times 600$ a ungerminated spore b spore in early stage of germination c germinated spore showing rapidly elongating sporeling

versicolor groups, they occur in scattered aggregates of varying conspicuousness, although no perithecia have been found. Occasionally, as in one of the *A. flavipes* group, foot cells and sterile cells which appear to be conidiophores ending in points instead of heads appear. These aborted conidiophores vary from nearly full length to vestigial. From the study of such material it is believed that Eddams' hulle cells represent aborted conidiophores surrounding the perithecia of *A. nidulans* and they may be indicative at least of a vestigial precursor of perithecium formation when they occur elsewhere.

Hulle cells do not, however, accompany the perithecia of either the *A. glaucus* group or *A. fischeri*. In the former the perithecia are smooth walled and naked, in the latter, they are surrounded by a loose network of sterile but unspecialized hyphae.

In *A. panamensis* (Raper and Thom 1944) aborted conidiophores commonly occur, but no hulle cells have yet been observed. In *A. unguis* (Thom and Raper, 1939), which is one of the *A. nidulans* group lacking only the ascospore phase the long sterile thick walled spores suggest homologous structures. To summarize, hulle cells are specialized structures that normally occur in certain groups of the Aspergilli. They are of somewhat questionable origin and in our experience are not known to serve any functional purpose. Schwartz (1928) figures the hulle cells of *A. nidulans* as capable of germinating and thus acting as reproductive cells. Since the cells of different groups are fairly characteristic, they often provide valuable diagnostic group and species characters.

Sclerotia

Definitely hard masses with characteristic surface marking and coloration and consisting of thick walled parenchyma like cells occur in several groups of Aspergilli. Such structures have not been seen in the groups typified by *A. clavatus*, *A. glaucus*, *A. fumigatus*, *A. nidulans*, *A. ustus*, *A. versicolor*, or *A. terreus*. On the other hand they develop regularly in certain members of the *A. candidus*, *A. niger*, *A. wentii*, *A. tamarii*, *A. flavus-oryzae* and in the *A. orchaceus* groups in other members of these same groups grown under similar conditions no such structures have been found. In occasional strains of *A. flavipes* dark hyphal masses are seen but these are not sufficiently compact to be considered true sclerotia. The development of sclerotia has not been followed in sufficient detail in any species to fix their genetic significance but the specialized characteristic structure of the sclerotia of many species lends color to the report of ascospore formation by Dangeard (1907) although no one else seems to have been able to verify such development. Environmental factors are known to influence sclerotium development but these have not been carefully analyzed.

CHAPTER IV

CULTIVATION AND EXAMINATION

An *Aspergillus* occurring upon a natural substratum may frequently be identified as to group from the original material if it belongs to one of the large and well marked groups. Among members of the same group however differences in the nature and composition of the substratum produce marked contrasts in colony appearance quantity of growth coloration measurements of fruiting structures and in the appearance of conidiophores and conidial masses. Many *Aspergilli* can be identified as to group from dried herbarium materials but the process of drying generally changes the colors materially and renders the hyphal masses so fragile that the morphological details necessary for identification inside a particular group are often obliterated or made difficult to interpret. Although accurate placing of such materials is sometimes possible, a much more satisfactory identification may be reached by transferring fresh material to culture media of known composition followed by purification of the cultures so that they present single species or strains for intensive study.

Two types of material for identification are regularly encountered by one undertaking a study of the *Aspergilli*: (1) the culture already isolated by another worker and (2) the moldy substratum with its natural flora of micro-organisms often representing several or many species including miscellaneous molds bacteria actinomycetes and even protozoa and other forms. In either case the final decision as to the proper name of an *Aspergillus* must usually be sought in fresh cultures made by the one responsible for identification.

Classification within the genus has become so dependent upon observation in pure culture that the whole subject of laboratory cultivation including favorable substrata culture making and culture handling needs to be considered.

CULTURE MEDIA

Pure culture upon known substrata is almost essential to the identification of *Aspergilli*. Since the morphological responses to diverse nutrients and especially to the stimulus of mixtures of other molds and bacteria growing with any particular species is great the study of each strain or species in pure culture in media of known composition is practically necessary.

Aside from Raulin's group in Paris most of the early culture work with *Aspergilli* was carried through upon so-called natural media. DeBary and

Brefeld used decoctions of horse dung variously diluted and stiffened with gelatin. Much of the European work was done with brewery wort, Mazé (personal communication 1904) used extract of white beans, potato and carrot decoctions have been widely used. Bainier grew his molds and described many of them upon sticks of licorice root, others preferred plugs of potato or of carrot, string beans, etc. The primary aim was to obtain an optimum growth of the mold under observation, rather than to analyze its relation to the substratum or furnish comparative data to distinguish it from other members of a series.

An optimum culture substratum for comparative study of the *Aspergilli* needs to contain the necessary chemical elements in the form of pure but assimilable salts supplemented by carbohydrates of such structure as to be available to the largest number of species, and purchasable in pure form in the chemical trade. Supplementary and often very important information must be sought from variations in the proportions of nutrients used or in the introduction of widely different substances in replacement of particular components of media already used. The *Aspergilli* as they are isolated from nature are not very dependent upon vitamins or growth promoting substances. For cultivation upon solid substrata agar is almost universally employed as a gelling agent.

When the study of a large number of molds is undertaken comparison of these molds under controlled and reproducible conditions of growth become essential. Foremost among the conditions which must be standardized is the culture medium or substratum. Various authors have proposed standardized and reproducible formulae which in their experience have provided uniform cultures over long periods; hence are of value for comparative studies. A series of such media are presented.

Czapek's Solution Agar

As a routine medium for comparative work the following formula originally adapted from Czapek (1902-1903) by Dox (1910) has been widely used. Minor variations in quantities apparently do not affect the reactions. Cultural information given in this manual is obtained from growth upon media produced by this formula unless otherwise specified.

Czapek's solution in ar

Water	1 000 cc
NaNO ₃	3 0 grams
K ₂ HPO ₄	1 0 gram
MgSO ₄ 7H ₂ O	0 5 gram
KCl	0 5 gram
FeSO ₄ 7H ₂ O	0 01 gram
Sucrose (Cube or other good commercial grade)	30 0 grams
Agar	15 0 grams

To reduce caramelization the sugar is added just prior to final sterilization.

Czapek's solution agar is not offered as an optimum substratum for any particular species but as a mixture approximately neutral in reaction which is readily made in any laboratory in fairly uniform manner, and which permits moderately vigorous growth of nearly all of the saprophytic *Aspergilli*. The quantities of mycelium and conidia produced by many forms are much greater upon other media but for comparative study a moderate growth of the majority of the species is more useful than the great mass of mycelium and conidia which are readily obtained by using enriched substrata.

Since the purpose of the Czapek formula is to insure the presence of the chemical elements required in quantities sufficient to support good growth it is frequently modified as to quantities and nutrients introduced. For some *Aspergilli* such as the *A. glaucus* group the addition of 20 percent or even 40 percent of sucrose has proved useful. Ammonium nitrate is sometimes substituted for sodium nitrate but with the loss of information as to whether the mold utilizes the ammonia or the nitrate or both. Dextrose is commonly substituted for sucrose. The monobasic potassium phosphate prevents precipitation of certain components in sterilization but it produces an acid instead of a neutral medium hence complicates many pieces of work. The introduction of peptone or yeast extract increases the sporulation of some forms. The same and other changes are made however by investigators who still refer to Czapek's solution as the basis of their work.

Biourge in his monograph of *Penicillium* (1923) and in his unpublished Manuscript of *Aspergillus* (1939), put much emphasis upon the method of preparing the neutral Raulin medium which he used for the growth of the colonies analyzed in making his species diagnoses.

Neutral Raulin's Solution—Dierckx Biourge

- 1 Magnesium carbonate 0.40 gram
Tartaric acid 0.71 gram
Triturate in a mortar with a few drops of distilled water and add quickly to a flask of distilled water make up to 100 ml.
- 2 To a liter flask with 800 to 900 ml. distilled water add

Sucrose	46.60 grams
Ammonium nitrate	2.66 grams
Ammonium phosphate	0.40 gram
Potassium carbonate	0.40 gram
Ammonium sulphate	0.16 gram
Zinc sulphate	0.04 gram
Iron sulphate	0.04 gram
- 3 Add 55 to 67 ml. of the magnesium tartrate solution (1) to the mineral salt sucrose solution (2) and make up to 1 000 ml. with distilled water.

In his detailed study of the *A. niger* group, Biourge's pupil, Mosseray (1934a) gives his simplified Raulin's solution as follows

Water distilled	1,000 ml
Sucrose	50 grams
Tartaric acid ¹	0.40 gram
Magnesium carbonate ²	0.250 gram
Ammonium nitrate ²	0.250 gram
Potassium carbonate ²	0.40 gram
Ammonium phosphate $(\text{NH}_4)_2\text{HPO}_4$ ¹	0.40 gram
Ammonium sulphate ¹	0.70 gram
Iron sulphate ¹ (cryst.)	0.05 gram
Zinc sulphate ¹ (cryst.)	0.05 gram
Agar agar ¹	20.00 grams

Sterilize 120° C for 20 minutes

Steinberg's Solution

Steinberg in the course of many years' investigation of a single strain of *Aspergillus niger* (NRRL No 334 Thom No 4247) developed a basic formula for testing other phases of the nutrition of his mold. It is called the "dibasic optimum" solution and carries mannitol instead of sucrose, sodium nitrite instead of ammonium nitrate, with the addition of sufficient sulphuric acid to obtain any desired reaction. Interpolations into this solution offer many possibilities.

Steinberg's dibasic optimum (Thom and Steinberg 1939)

Water (distilled in Pyrex still)	1 000.0 grams
d Mannitol	50.0 grams
NaNO ₂	2.0 grams
K ₂ HPO ₄	0.35 gram
MgSO ₄ 7H ₂ O	0.75 gram
FeSO ₄ 7H ₂ O	0.001 gram
ZnSO ₄ 7H ₂ O	0.00088 gram
CuSO ₄ 5H ₂ O	0.00020 gram
MnSO ₄ 4H ₂ O	0.00012 gram
NaMoO ₄ 2H ₂ O	0.00005 gram
H ₂ SO ₄ to pH 4	

A number of so-called natural substrata including malt extract and hay infusion agars are very useful in the study of the *Aspergilli*. A great majority of species sporulate more freely upon malt extract than upon Czapek's solution agar (fig. 9), and for this reason it is very useful where large quantities of spores are desired. This medium is however less diagnostic than Czapek's solution.

¹ Merck reagents

² Kahlbaum reagents

Malt Extract Agar (Blakeslee's Formula 1915)

Distilled water	1 000 cc
Malt extract	20 grams
Peptone	1 gram
Dextrose	20 grams
Agar	20 grams

Add dextrose just prior to final sterilization. Conidial structures are generally more numerous and are often borne on shorter conidiophores and there is an almost complete absence of coloration in the substratum. Except in a few isolated cases coloration of the spore heads themselves is not materially altered. While the production of exudate in the form of drops is not characteristic of many of the *Aspergilli*, it can be generally said that droplet formation on malt agar is much less than upon Czapek's solution agar.

Hay infusion agar is very useful in the isolation of *Aspergilli* from nature. A 1:10 suspension of soil in sterile water is streaked on hay infusion agar plates and incubated for one week to 10 days. Isolations are then made from individual fruiting structures with the aid of a low power binocular.

Hay Infusion Agar

Distilled water	1 000 cc
Decomposing hay	50 grams
Autoclave for 15 minutes at 15 pounds	Filter
Infusion filtrate	1 000 cc
K_2HPO_4	2 grams
Agar	15 grams
Adjust pH to 6.2±	

No *Aspergillus* makes a luxuriant growth upon this medium, but a great variety of forms make a limited development. Furthermore, such fruiting structures as are produced are generally characteristic of the different species present. It thus constitutes a very favorable substratum with which to analyze and isolate the *Aspergilli* occurring in soils or other natural substrates. The medium is likewise useful for securing limited sporulation of certain forms, such as *Aspergillus sparsus*, which fruit very sparsely upon Czapek's solution agar.

Czapek's solution agar enriched with peptone or corn steeping liquor is often very useful. For example, in the cultivation of members of the glaucous group, the addition of a limited amount of peptone greatly increases the production of conidia, while addition of a small amount of corn steeping liquor (e.g. 0.2 percent) increases the growth of most forms without markedly affecting the character of the resulting colonies.

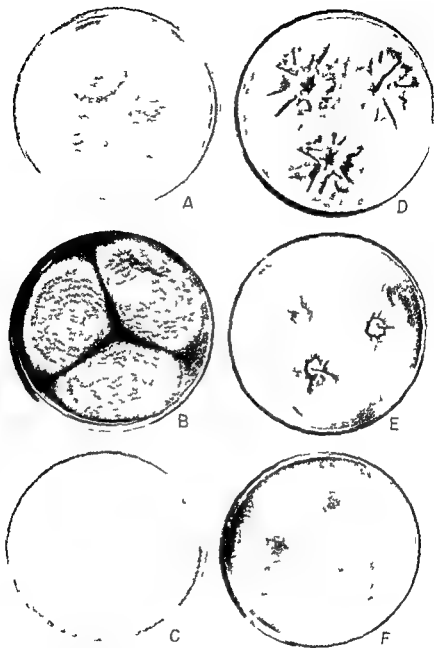


FIG. 11 Influence of substratum. A-C *Aspergillus varicolor* NRRL No. 217 growing upon Czapek's solution malt extract and hay infusion agars respectively 10 days room temperature. Note particularly the heavy development of perithecia upon malt and the very sparse development of the same upon hay infusion agar. D-F *Aspergillus coespitosus* NRRL No. 1929 growing upon the same media under similar conditions. Upon Czapek's solution agar (D) dark hülle cell masses develop along with a limited development of conidial structures upon malt (E) and hay infusion (F) agars hülle cell masses are lacking and conidial structures are very abundant.

SPORULATION MEDIA

In addition to the ordinary handling of molds in the laboratory exploration of their biochemical potentialities often necessitates the production of considerable quantities of spores. Whereas some of the media listed above may be employed for this purpose, it is generally advisable to employ so-called sporulation media. Requirements of different species and strains vary and one frequently has to develop special solutions to meet the needs of particular organisms under study. Such media may be used either as liquid substrata or as solutions solidified with agar. In either case the objective is the same—to secure the maximum production of spores with the production of as little vegetative mycelium as possible.²

A number of sporulation media for use with different molds have been developed by Dr. A. J. Moyer. Three of these will be cited while additional formulae may be found in the papers published by members of the Fermentation Division, Northern Regional Research Laboratory.

*Sporulation medium for A. niger (Moyer, Wells, Stubbs,
Herrick and May 1937)*

Glucose	51.3 grams
NH ₄ NO ₃	1450 gram
KH ₂ PO ₄	0.01 gram
MgSO ₄ · 7H ₂ O	0.000 gram
Beer	60.0 ml
Distilled water to make	1 liter

This solution can also be used as a solid medium by the addition of 0.5 gm. per liter CaCO₃ and 30.0 gm. per liter agar. The above solution was subsequently modified as follows (Gastrock, Porges, Well and Moyer 1938):

Glucose	50.0 grams
(NH ₄) ₂ HPO ₄	0.500 gram
KH ₂ PO ₄	0.144 gram
MgSO ₄ · 7H ₂ O	0.170 gram
Peptone	0.70 gram
Beer	45.0 ml
Distilled water to make	1 liter

² For the surface inoculation of nutrient solutions in small flasks or other containers, dry spores in quantity can be removed from agar surfaces and floated on the liquid surface by means of a 5 mm. loop. To inoculate from a liquid culture the usual procedure is to remove a small portion of the heavily sporing mat, transfer this to the solution, and dislodge the spores by vigorous agitation. Spores from either type of culture can be suspended in water and used as inoculum in submerged or shaken cultures. The addition of sodium lauryl sulfonate to the suspending water in a concentration of 1:10,000 aids greatly in securing uniform spore suspensions.

Sporulation medium for A. flavus (Moyer, Personal communication)

Glucose (commercial)	165.0 grams
Bacto Peptone	1.0 gram
MgSO ₄ 7H ₂ O	0.050 gram
KH ₂ PO ₄	0.060 gram
KNO ₃	0.500 gram
Fe (as tartrate)	0.040 gram
Agar	0.25 gram
Distilled water to make	1 liter

The above solution can be used as a solid medium by increasing the agar concentration to 30.0 gm per liter (The small amount of agar included in the basic formula is added solely for the purpose of increasing viscosity). Incubation should be at 22° to 24° C. This factor is critical for maximum spore production with the kojic acid producing strain, NRRL No. 484 (Thom No. 3538) for which the medium was developed. This medium can also be successfully employed for spore production in *A. niger* by incubating cultures at 30° C.

Abundant sporulation of many strains and species can be secured by cultivation upon bread, whole cereal grains, or various types of milled products of the same. Bread, if used, should not contain propionates or other mold inhibitors. The material to be inoculated should be moist but in no sense wet, and special precautions must be taken to insure that the grain or bread is properly sterilized before being used. The use of grain as a substratum for molds dates back to prehistoric time in the fermentation industries of the Orient where rice was and still is, commonly used to produce the "koji," or inoculum used in the alcoholic and soya fermentation industries of that area. In its classic usage, the molds cultivated were mostly members of the *A. flavus oryzae* group but experience has shown that this general type of medium can be used to advantage to secure heavy sporulation of many other forms. From such material series of surface fermentation flasks or other vessels can be uniformly inoculated by various means involving aspiration of spores or the direct transfer of heavily spore laden particles. Spore suspensions can be prepared and used for seeding submerged cultures.

TYPES OF CULTURE

Cultures for grouping and identification of the Aspergilli should be grown in petri dishes. At the same time an adequate number of slanted tubes should be inoculated and held in reserve as an uncontaminated stock culture. Colonies so situated in the petri dish that they can be viewed directly under low magnifications with the compound microscope are necessary to supply a clear picture of the structure and course of development of mycelium and fruiting parts. Such colonies can be obtained by several

procedures some of which have been developed for special tests or for individual mold problems. Practices useful in bacteriology are often applicable to the problems of the mycologist. Selection of a procedure which satisfactorily provides the information necessary to describe an *Aspergillus* calls for discussion of a series of procedures commonly in use. Only in that way may we show why some of these are adapted to the problems of identifying an *Aspergillus* while others fail for specified reasons, to furnish important observations.

Spot Inoculations

Over long periods and in the hands of many investigators some type of mass conidial inoculum has given dependable and reproducible results. The most common method of transfer and on the whole probably the most satisfactory one for maintaining a strain of *Aspergillus* as well as other molds is the removal from the stock culture of a variable mass of conidia, fruiting structures or vegetative hyphae with some sort of needle or loop and the transfer of this material to selected positions on fresh medium. Practices differ. With the *Aspergilli* colonies so placed as to permit radiate development from the point of inoculation are most satisfactory for study. Where there are two or three colonies to the plate these eventually reach into each other's zones of influence. They may blend and become indistinct or inhibit each other and leave sterile bands between the colonies. Both types of culture furnish useful data. Usually, the line where two colonies approach and partially or completely inhibit each other hastens fruiting in the adjacent margins and permits favorable examination with the compound microscope. The opposite margin of the same colony unaffected by competition furnishes at the same time the normal and symmetrical growth which is typical of the species. The one-colony plate usually provides the most striking exhibit of the species but the 2 or 3-colony plate is the most generally useful (fig. 10).

Once an *Aspergillus* has been obtained in pure culture the most effective way to insure that plates will contain 1, 2 or 3 colonies as desired is to suspend a quantity of spores in melted agar at approximately 45° C. allow this to solidify and then transfer small quantities of the gelled suspension to the surface of plates or agar tubes in the positions where the colonies are desired. With the *Aspergilli* as with all of the molds which produce dry spores it is often very difficult to secure placement of colonies at selected positions and only at such positions without the use of some type of wetted inoculum.

In the routine examination of *Aspergilli* where it is not essential that the number of colonies be limited to 3 or less satisfactory transfers have been made by using a sharp nichrome wire as an inoculating needle and selecting

the inoculum from a colony in a petri dish by working under a 10 \times pocket magnifier, or under a binocular microscope of the Greenough type carrying similarly magnifying lenses. Material to be removed can be exactly located in the parent colony. It is found possible in dealing with most *Aspergilli* (1) to remove conidia from a single head, (2) to remove one or more heads borne upon a single hypha at the margin of the colony, or (3) to select vegetative hyphal tips from a single mycelial sector. It is usually possible to avoid (1) heads and conidia of other species or strains (2) foreign mycelia and (3) bacterial contaminations present in the substratum. Purity in repeated culture over many years has been possible by this procedure.

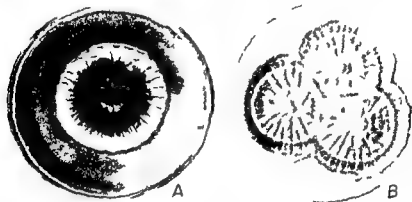


FIG. 10. Single and three point cultures of *Aspergillus foetidus* on Czapek's solution agar room temperature 10 days $\times \frac{1}{2}$ approximately. Note that spore production extends to the colony margins in the central triangle of figure B and almost to the outer colony margins as well. In figure A sporulation is limited to the central area only. Figure B is favorable for direct examination with the low power objective of the compound microscope; figure A is not.

Dilution Cultures

If dilution cultures are to be used the suspension of conidia should be sufficiently dilute so that the individual petri dish will show not more than six but preferably not over three colonies. Such dilutions are difficult to gauge and often result in some plates crowded with numerous colonies while others contain none. The colony locations are not under control. In general, the dilution of spores in successive water blanks with the subsequent plating of aliquots from such dilutions has not been found satisfactory.

An alternate technique involves the introduction of one loopful of inoculating material into a tube of melted agar which is then rolled or shaken

and a loopful removed to a second tube followed by the same manipulation a third or fourth time. The higher dilutions are then poured into petri dishes where colonies develop. This method has all the faults of the water blank dilution technique. The procedure has been widely used but has not been followed in our study of the *Aspergilli*. Blakeslee (1915) and other mycologists have prepared such dilutions in bottles by slowly rotating the bottle in a cold water or ice bath, thus allowing the agar to congeal in a thin layer against the glass surface. This practice has little to recommend it (1) the resulting colonies could be isolated more readily from a petri dish, and (2) it is basically impractical to make satisfactory observations regarding the character and structure of a mold colony or the gross features of its fruiting structures when observations have to be made through the curved and non uniform walls of a glass bottle.

Smears and Streak Cultures

The practice of smearing a suspension of mold spores over the whole plate or the whole length of a slanted agar tube results in a growth of mycelium which covers the entire surface and usually produces a greater mass of conidia. But individual colonies are usually unidentifiable in such preparations hence they are of little value in providing those critical details of colony habit, coloration and texture necessary for identification and classification. In general mycelium derived from different conidia of the same strain will intertwine without inhibition and even anastomose if they come into contact in the early stages of growth for example before any sign of conidium production appears. If the spacing of the spores is great enough to permit the establishment of small fruiting colonies before such contact is made the colonies frequently do not converge and complete coverage of the surface of the agar with the production of maximal quantities of conidia does not occur. Closely placed seeding of spores is useful to obtain the largest possible supply of conidia but to study the normal characters of a species individual colonies must be allowed to develop without interference by others of the same or different species.

Streak cultures can be employed to advantage in freeing one mold from another or from other contaminating organisms. By touching a sterile moistened needle to a single conidial head or other selected fruiting surface of limited extent and subsequently streaking this repeatedly across the surface of an agar plate isolated colonies of the desired species can usually be obtained. Occasionally it is desirable to streak two plates in succession in order to secure a satisfactory separation of colonies. In any case the spore source should be selected with great care and the use of a low power binocular microscope or pocket magnifier is recommended. When this method is employed it is desirable to reisolate from these individual colo-

nies as soon as possible after sporulation begins. For freeing one species or strain of *Aspergillus* from another, or from a contaminating *Penicillium*, streaking upon Czapek's solution agar usually gives satisfactory results. The method finds its greatest usefulness in separating comparatively slow growing molds, such as the *Aspergilli*, from such very rapidly growing forms as the *Mucoraceae*, *Trichoderma*, etc. The critical step in this procedure is to isolate the slow growing *Aspergilli* during the first two to three days before the whole plate is overrun by the spreading faster growing forms. If the contamination is bacterial in nature malt extract agar or some other medium of more acid reaction should be substituted. Diluting the spores in water before streaking is not recommended since this often tends to disperse the contaminating organism more than it does the desired form, this is especially true with bacterial contaminations actinomycetes, and other minute forms.

Single Spore Cultures

The isolation of cultures from single spores is a time-honored technique with many mycologists and with many workers it is considered a "must." When properly employed, there is much to recommend this practice. There are also certain dangers inherent in this procedure hence we believe it worth while to consider the subject at some length and to analyze both its advantages and its limitations. As generally employed, the primary objective of single spore isolations is to secure cultures of unquestionable purity. If the operation is skillfully performed and adequately verified there can be no doubt but that the resulting colony will represent a pure culture of a single species or strain. The continued purity and physiological stability of such cultures however cannot be taken for granted. There is no substitute for vigilance, and the culture thus isolated must be kept under critical observation.

As a means of purifying a culture i. e., separating it from foreign forms, the single spore method undoubtedly has its place but it cannot be recommended as a means of preserving the morphological and physiological stability of a particular strain. One has only to study the reports of Stakman and his associates to realize that monospore selection and isolation is not a touchstone to strain stability. In fact they have ably exploited the technique to show just the opposite. While we do not have an accumulation of data on the *Aspergilli* and related genera which can compare with that on the smuts and other pathogenic fungi we do have enough information to know that one of the best methods of obtaining change in some of the *Aspergilli* is to make a series of successive monosporous isolations. For every species and strain there is apparently a normal range of natural variation and by isolating single spores it is often possible to secure certain

progeny which represent essentially the outside limits of such variation. This is worth while since it offers one means of securing strains which may prove more useful than the parent culture for some particular purpose, industrial or otherwise. The single spore method can be of real value in purifying a culture. It can be of real value as a means of dissecting a culture. But once a promising strain has been discovered and its purity is established, perpetuation by the transfer of masses of conidia is the best safe-guard for preserving in a constant condition, its morphological and physiological characteristics.

Some of the techniques by which such single spore isolations can be made follow.

(1) *Serial Dilution*. Spores are thoroughly suspended in water and the resulting suspension is subsequently diluted in sterile water blanks in steps of 1:5 or 1:10. One cubic centimeter aliquots from two or three selected dilutions depending upon the density of the original suspension are added to sterile petri dishes. Melted agar at approximately 45°C is then added to these plates which are rotated to secure uniform mixing of the still liquid agar and the diluted spore suspension. The plates are then incubated at room temperature and isolates are made from plates showing a limited number of colonies which are uniformly separated. Using this technique one cannot be certain that any particular colony results from a single spore but if the original suspension was properly prepared one can feel sure that more than 95 percent of the colonies resulted from single spores. More uniform suspensions of spores can be obtained by adding to the suspending medium some suitable detergent or aerosol. For this purpose we have successfully used sodium lauryl sulfonate in concentrations of 1:10,000 or 1:100,000 without apparent harmful effect upon the molds under study. The dilution method of securing single spore isolations is more rapid than any other and for many purposes it is quite satisfactory.

(2) *Selection and Removal of Individual Spores*. Where the investigator wishes to be positive that every colony results from a single spore it is necessary to employ some technique combining actual microscopic examination with some device for the mechanical removal of selected spores. The various types of micro-manipulators are well suited for this work and with sufficient practice single spore isolations can be made quite satisfactorily with any of these. Dilute spore suspensions in water are mounted on the undersurface of a cover slip supported by a glass chamber open at either one or both ends. With the aid of mechanical controls and a micro-pipette a single spore is withdrawn from the suspension and ejected upon a suitable substratum where the spore develops into a mature colony.

Single spores can likewise be removed by mechanical cutting devices such as those described by LaRue (1920), Keitt (1915) and Lambert (1939).

A comparatively thin spore suspension is spread on an agar surface and single well-separated spores are located with the microscope. A small cutting device, mounted on a holder screwed into the nose-piece of the same microscope is lowered into the agar and a small block bearing the selected spore is removed. This is subsequently transferred to a suitable culture surface where the colony develops.

At the Northern Regional Research Laboratory we have employed a somewhat different and simpler method. A thin spore suspension is spread evenly over the surface of a firm agar gel that has been specially filtered to remove all particulate matter. This is incubated overnight and the spores allowed to germinate. On the following day well separated sporelings are



FIG. 11. Single spore isolation. A A single well isolated germinated conidium of *Aspergillus niger*. B The same removed on a small agar block and transplanted to a fresh agar plate as described in the text. $\times 41 \times 300$

located with the aid of a microscope and their positions marked on the under surface of the culture dish. The area is then checked with a 8 mm objective and 10X or 15X oculars to insure that there are no other ungerminated spores in the same area. Using a wide-field binocular of the Greenough type and very small micro scalpels fashioned out of platinum iridium wire (B and S gauge 22 or 24) small agar blocks on which the spores rest are transplanted to fresh agar plates. Each of these agar blocks is then examined again with the 8 mm objective to insure that the selected spore has been transplanted. An experienced worker can isolate from 25 to 30 spores within a period of an hour. Photographs showing essential steps in this technique are presented in figure 11.

Hanging Drop Cultures

The drop of culture fluid inoculated with conidia and hanging from a slide or cover glass into a closed chamber can be incubated and examined readily. It furnishes information as to the percentage of conidia which are viable, the changes which occur in the germinating spore such as swelling, bursting along definite lines, germination from specialized germ pores, or branching of the germ tube, but descriptions of fruiting structures in such hanging drops are worthless in the study of the mold colony or normal fruiting habits of the species.

TEMPERATURE

The great majority of *Aspergilli* grow well and sporulate abundantly at temperatures of 20 to 26° C. For this reason, most cultures can be incubated on laboratory tables or shelves, and it is not necessary to give special consideration to incubation. There are, however, certain exceptions. The large-pored members of the *A. pergillus glaucus* group such as *A. echinulatus* and *A. nullo-glaucus* grow more rapidly and fruit more abundantly at 20° than at 21 to 25° C. (Thom and Raper 1941). This temperature response is especially marked in *A. medius*; at 18 to 20° C. growth is rapid, abundant, large conidial heads are produced, and numerous perithecia are developed; at 25° C. and above growth is restricted, few and smaller conidial heads are developed, and only occasionally perithecia are produced (fig. 12). On the other hand, the very abundant small-spored members of the *A. glaucus* group such as *A. repens*, *A. chalcidii*, and *A. amstelodami* grow rapidly and fruit abundantly at 30° C. (Thom and Raper 1941). In *A. janus* (Raper and Thom 1944) two different types of conidial heads are produced, and the ratio of the two types is strongly influenced by temperature (fig. 12): at 18 to 20° C. almost all heads are white with clavate vesicles and are borne upon long conidiophores; at 30° C. almost all heads are dark green with globose vesicles and are borne upon short conidiophores (see species description, p. 187).

When grown upon suitable media and incubated at 20° C. in the presence of light or alternate light and darkness, *Aspergillus giganteus* produces large heads on long conidiophores ranging up to 5 or even 10 cm. In similar cultures incubated at 30° C. heads are smaller and conidiophores are uniformly short, rarely exceeding 1 cm. in length, whether the cultures are exposed to light or incubated in total darkness. *A. terreus*, *A. carneus*, and *A. fischeri* thrive at temperatures up to 35° C. while *A. fumigatus* grows well at 45° or even 50° C. In all of these forms growth is more rapid and sporulation more abundant at 30° C. than at normal laboratory tempera-

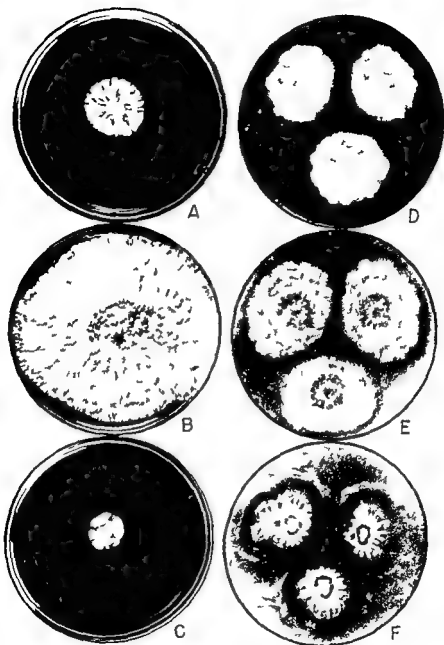


FIG 12 Influence of temperature upon growth and sporulation in *Aspergillus medius* NRRL No 124 and *Aspergillus janus* NRRL No 1787 A B and C *Aspergillus medius* 17 days at 12 C 20 to 22 C and 30 C respectively note maximum growth and limited sporulation at 20 to 22 C D E and F *Aspergillus janus* 3 weeks at 20 C 24 to 25 C and 30 C respectively note evidence of white heads only at 20 C abundance of white and presence of green heads at 24 to 25 C and green heads only at 30 C Temperature is the only variable

tures of 24° to 25° C, although wholly typical cultures are produced at both temperature levels

In the production of spores for biochemical investigation (see p 37) and in the conduct of such studies themselves temperature is often very important In work of this kind it is essential to determine the optimum temperature for each organism and process, and then to control this within a narrow range in order to secure consistent and reproducible results

Temperature is known to be a critical or limiting factor only in a few species but its effect in these is sufficiently pronounced that its influence should not be disregarded in any case

CULTURE TOOLS AND EQUIPMENT

Transfer Needles

In making transfers we have used with satisfaction for many years a No 20 or No 22 B and S gauge nichrome wire thrust into a slender brass or stainless steel tube so that only about 15 to 20 mm are exposed (The tubing employed is of the type used in temperature controls of mechanical refrigerators and other thermostatically controlled devices) The point of this wire is then ground down to the sharpness and smoothness of a needle This instrument can be heated to redness in the flame a great many times with only the occasional necessity of resharpening It thus has many advantages beside cheapness it is firmer and takes a better point than platinum it withstands sterilization in the flame which promptly destroys the usefulness of steel and it can be made any size or length to suit the workman's purposes or preferences

Loops

Loops of various dimensions are very useful and can be inserted into handles made from small brass or stainless steel tubing as noted above These too can be fashioned from nichrome wire but it has been our experience that loops made of platinum iridium wire possess certain marked advantages While these are not as rigid as nichrome loops they are much more rigid than loops of pure platinum and they can be re-heated indefinitely without corroding Loops of this type will be found especially useful in making mass inoculations such as the seeding of large tubes and plates for the production of spores to be used in various types of experimental work

Mounting Fluid

In the microscopic examination of the *Aspergilli* it is often satisfactory to mount conidia heads or other structures in water It is more generally

satisfactory, however, to use some mounting fluid of a composition designed neither to swell nor plasmolyze the tissues to be observed. Such a mounting fluid was developed by Amann as early as 1896 and has been used by mycologists, quite generally, for many years. Its composition is as follows:

Carbolic Acid Crystals (c p)	20.0 grams
Lactic Acid (sp gr 1.2)	20.0 grams
Glycerine (sp gr 1.25)	40.0 grams
Distilled water	20.0 cc

The carbolic acid crystals are first liquefied by heating in a water bath.

The mounting fluid is normally used without the addition of any dye since in the diagnosis of the *Aspergilli* the natural colors of conidiophores, conidia, etc., are very important. If it is considered desirable to stain the tissues under observation, it is possible to incorporate into the lactophenol solution some coloring substance such as cotton blue, eosin, or some other aniline dye. In making mounts of the *Aspergilli* it is profitable to wet the material first with 70 percent alcohol to drive off air bubbles and then quickly add a small drop of the lactophenol prior to the placement of the cover glass.

Incubators

Almost all of the *Aspergilli* grow well and sporulate abundantly at laboratory temperature. There are, however, certain exceptions to this general rule (see p. 45), and for this reason it is desirable to have available an incubator which can be regulated at temperatures below that of the laboratory and others covering different ranges up to 37° C. or even 50° C. The type of incubator is not critical and almost any type of cupboard or room will prove satisfactory if the temperature can be controlled to within 1° C. \pm and if the air is neither excessively dry nor humid to the point where cotton plugs become moist upon continued exposure.

Microscopes

In the study and identification of *Aspergilli* it is essential to have at one's disposal a good-quality compound microscope. If possible this should be provided with apochromatic lenses. In our experience we have found a 3 mm. objective used in conjunction with either 10X or 15X oculars to give us magnifications and a degree of definition which is most satisfactory for the examination of conidial structures. While it is not absolutely necessary it is desirable to have in addition a low power wide-field dissecting microscope covering magnifications from 10 to 40 diameters. In the absence of such a microscope a high quality pocket magnifier provides a good substitute.

Photographic Equipment

A limited amount of photographic equipment is a very valuable aid in the study of this or any other group of molds. A camera should be available which can be used in conjunction with a compound microscope and to which low power lenses can be attached directly. In addition to a good quality lens producing pictures above and below natural size a series of Tessar lenses which will provide magnifications of from 5 to 30 diameters is extremely useful. With the aid of these details of colony structure can often be recorded which cannot be pictured with the lenses of the compound microscope and which are very difficult to describe adequately in words. With these low power lenses it is possible to photograph types of fruiting heads, the relative abundance of conidial structures to vegetative mycelium, and the relationship between each of the above and sclerotia or perithecia when such structures are present.

CHAPTER V

PRESERVATION OF CULTURES

Any mold that is valuable because it has been used in fundamental research work, because it has been found useful in some industrial process, or because it is a significant agent in some destructive or pathogenic situation should be preserved to insure its identity for subsequent use or reference. Identification by description will take the careful worker to the group species, and often to the variety as based upon morphology or some conspicuous character, but the reidentification from description of the exact organism used in a biochemical investigation or discovered in some ecological situation is generally impossible. Culture collections have therefore, developed. The Centraalbureau voor Schimmelcultures (cited by them as CBS) at Baarn, Holland, the National Type Culture Collection in London, and the American Type Culture Collection at Washington are well known sources of such material. More recently established but containing a greater number of industrially important molds is the culture collection of the Northern Regional Research Laboratory (commonly cited as N R R L) at Peoria, Illinois. The *Aspergilli* are especially well represented and contained in the collection are almost all of the species considered in this manual together with records which check the identifying numbers of this collection against the records of the source collections* which were brought into it.

In undertaking a comparative study of the *Aspergilli*, or any other group of molds it is essential to maintain in a viable state a large number of isolates representing diverse species and strains. By this means it is often possible to interpret current isolates and accessions in terms of historic types and concepts. In maintaining such a collection of molds the objective should be to preserve viability without growth or germination of spores during the storage period. By so doing the user can reasonably expect to maintain his organism without variation, degeneration or mutation. A number of techniques can be employed to advantage the more useful of which will be considered in some detail with certain of their advantages and limitations noted.

Currently contained in this collection are the molds formerly maintained by Thom and Church and later by Thom and Raper, in the United States Department of Agriculture, Washington, D. C., many of the *Mucorineae* maintained by Dr. A. F. Blakeslee for many years at the Carnegie Institution, Cold Spring Harbor, Long Island, New York, a large number of miscellaneous forms from the Harvard University Collection initiated by Prof. R. Thaxter and more recently maintained by Dr. D. H. Linder, and limited numbers of cultures from many other collaborators.

Agar Slant Method

The method most generally employed for maintaining mold cultures and the one which has been successfully used by the writers for many years, may be termed the agar slant method. This involves the periodic transfer of spores from old agar slants or plate cultures to new agar tubes. The composition of the substratum is varied to suit particular requirements and groups of organisms. In our work we regularly employ the Czapek's solution agar. Few if any strains make their maximum growth on this medium but it has been our experience that they maintain exceptionally well any characteristic morphological or physiological features which may characterize them. It is necessary to know the expected viability of all cultures to be maintained and to gauge the intervals of transfer accordingly. With the *Aspergilli* transfer every 8 to 9 months is sufficiently frequent for all species with the possible exceptions of *A. citrisporus* and *A. staccinicus* and a period of one year is not too long for most forms. In practice it is advisable to handle separately the few very short lived species and to set the regular period of transfer well within the known viability period of the remaining forms. Transfer of the general collection at least once each year insures a complete survey within the yearly period of all strains maintained. New tubes are inoculated at least in duplicate while triplicate preparations afford a desirable margin of safety. The new cultures are incubated for 2 to 3 weeks or until a good crop of spores has developed. Incubation at room temperature is suitable for most of the *Aspergilli* although a few forms such as the large-spored members of the *A. glaucus* group sporulate more abundantly at 20° C. The correctness of the cultures is then checked with a wide-field binocular or a 10X pocket magnifier the plugs are poisoned to preclude any possibility of subsequent contamination and selected tubes are placed in storage. Cultures can be stored for reasonable periods at room temperature certain species will remain viable for many years at 24 to 26° C. The viability of most species is materially lengthened and the possibility of progressive variation reduced by storage at 2 to 4° C (i.e. above any danger of actual freezing but sufficiently low to prohibit further growth and possible dissociation). Tubes of any desired size may be employed. We have found lipless tubes 15 by 125 mm to be quite satisfactory since they provide adequate culture surface and at the same time require much less storage space than the larger tubes commonly in use. Each culture should be maintained at least in duplicate with the different tubes of each pair stored in separate refrigerators. With the accidents and failures of refrigeration the possible escape of toxic gases or the possible ingress of contaminations that escape the usual inspection the maintenance of not less than two complete series of strains is a necessary precaution. If natural conditions such as temperature and relative hu-

midity are favorable as at Baarn Holland, refrigeration may be dispensed with, but watchfulness against invasion by mites becomes more important. Additional slants should be prepared for cultures which are frequently used.

Agar slant cultures are convenient for use, easily examined and compared, and easily replaced (fig 13 A). They are, however easily contaminated when handled carelessly. Uneven drying subjects the culture to

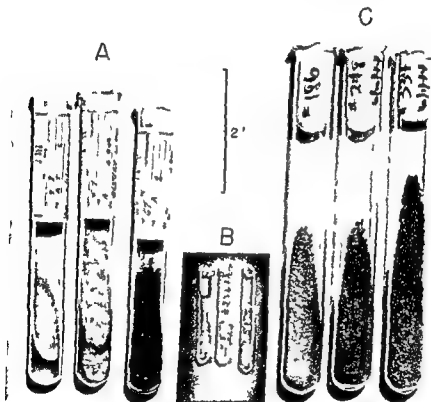


FIG. 13. Methods of maintaining stock cultures as discussed in the text. A. Cultures growing on agar slants. B. Cultures preserved in lyophilic form; note the compact, chalky pellets formed by the dried serum in which the spores are suspended. C. Cultures preserved in dry soil.

extremes of contrast in concentration of media and metabolic end products between the thin edge of the slant and the heavier mass in the bottom of the tube. Variations (apparent or real) often appear in the stored cultures and these may be propagated in subsequent transfers. As a safeguard stock cultures should be grown in petri dishes after several transfers in tubes thus making possible more complete examination to maintain purity and typical morphology.

Preservation in Lyophilic Form

Studies now in progress at the Northern Regional Research Laboratory indicate that many if not all of the *Aspergilli* can be successfully maintained in a dried state for extended periods. Viability tests for a number of species including *A. terreus*, *A. niger*, *A. oryzae*, *A. flavus* and *A. glaucus* have been made at 3½ years while a much greater and wider variety of forms has been tested at 20 to 24 months. Positive results have been obtained with all cultures tested although comparatively few colonies developed from certain strains of *A. niger*, *A. flavus* and the large-spored members of the *A. glaucus* group. Observations are being continued and in time information will be obtained as to the feasibility of employing this as the principal means of maintaining a collection of molds. It is known that many bacteria especially staphylococci, streptococci and pneumococci can be successfully preserved for periods up to 16 to 18 years (Elser, Thomas and Steffen 1935; Swift 1937). Wickerham and Andreasen (1942) have presented evidence covering a period of one year which suggests the practicability of applying the method to the yeasts. Such information as we have to date regarding the molds seems to indicate that the method may prove of great significance in two ways: first as a means of prolonging viability and second as a means of preserving in viable form spores of a particular generation or other selected origin which can be used in comparative tests over a period of many months or even years.

The drying technique employed at the Northern Regional Research Laboratory is essentially like that described by Wickerham and Andreasen (1942) and may be briefly summarized as follows:

Employing aseptic techniques throughout the spores from selected cultures are suspended in sterile beef or horse serum. The resulting suspension is then dispensed into small cotton stoppered Pyrex glass tubes 8 mm by 100 mm that have been properly labeled with glass-marking ink. Approximately 0.05 to 0.1 cc of the spore suspension is added to each tube by means of a long thin necked pipette. Most of the cotton plug is burned away and the remaining portion pushed down into the tube to prevent possible contamination during the drying process. The tubes are inserted in rubber sleeves on the manifold as shown in figures 14A and 14B and lowered into a freezing bath of carbon dioxide ice and methyl cellosolve at a temperature of approximately -40°C. The suspension is frozen almost instantaneously. The manifold is connected to a vacuum pump and evacuation and desiccation initiated. Water is removed from the system by the insertion of a water trap immersed in a CO₂-methyl cellosolve filled Dewar flask as shown in figure 14A or in a column of drierite (anhydrous CaSO₄) as shown in figure 14B. After a few minutes the temperature of the bath

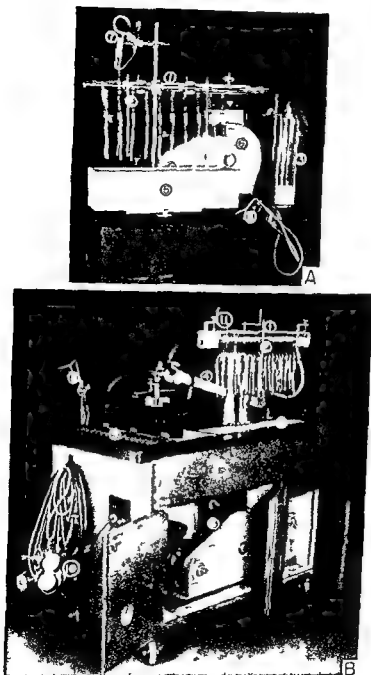


FIG 14 Apparatus employed at the Northern Regional Research Laboratory for preserving microorganisms in lyophilic form A Table model of thirty tube capacity utilizing a trap immersed in a Dewar flask filled with CO_2 ice and methyl cellosolve to collect the water vapor removed from the drying preparations B Larger self

PRESERVATION OF CULTURES

surrounding the tubes is raised to approximately -5°C by supplying additional methyl cellosolve that has been precooled to approximately -10°C . The bath is maintained at this level continuously until the preparations appear thoroughly dry. In drying the serum contracts slightly to form a well-defined chalky pellet. When the pellets are apparently dry, the tubes are raised above the bath and evacuation is continued for one-half to three-quarters of an hour at room temperature to insure as complete removal of water as possible, after which time they are sealed off with a gas-torch (fig. 14 A). On the following day each tube is tested for the presence of a good vacuum by means of a high frequency spark coil tester, and those tubes which show such a vacuum are retained. Tubes not making a satisfactory vacuum are very rarely encountered in actual practice. Quadruplicate tubes are regularly made for each stock culture, and finished preparations are stored in a refrigerator.

In recultivating the molds, the tubes are marked with a file scratch, flame-sterilized and the tube broken inside a wrapping of sterile paper. The content which is in the form of a well formed pellet (fig. 13 B), is dissolved in 1 to 2 cc. of sterile broth or water. This is streaked on agar plates and colonies are allowed to develop. New isolations can be made within a period of a few days. It is possible of course to go directly from the lyophile tubes into flasks or other cultures used in actual experiments but generally speaking much larger quantities of material would have to be processed.

The feasibility of preserving molds in lyophile form over long periods has by no means been proved but results to date are very encouraging. Should it be found that spores of molds like bacterial cells can be preserved viable by this method for many years it will prove ideal as a means of preservation.

¹ Wickerham and Andreasen in 1942 governed the temperature at which the preparation was dried by adjusting the level of the tubes above a bath which was at a very low temperature. Subsequent to this Wickerham developed the procedure outlined above.

contained and portable unit of sixty tube capacity (designed by Dr. L. J. Wickerham) which utilizes a column of anhydrous calcium sulphate (drierite) to collect vapor removed from drying preparations. A, and B, Glass manifolds. C, Thermometer. D, Dewar flask containing water vapor trap immersed in ice and methyl cellosolve. E, Column of drierite. A, and B, Freezing bath containing CO₂ ice and methyl cellosolve in which preparations are immersed for quick freezing and subsequent temperature control. A, and B, Vacuum pump. Vacuum gauge (mounted at opposite end of apparatus shown in figure B). A, Gas-oxygen torch for sealing off dried preparations. B, Terminals on which tubes are mounted to be tested for presence of good vacuum by means of a high frequency spark coil tester (not shown). B, Oxygen tank. B, Screw lift for raising and lowering manifold and attached tubes (in figure 4 manifold is raised and locked manually and locked into position by means of a wing bolt).

serving large culture collections. It possesses certain marked advantages

- (1) There is no possibility of contaminants entering the sealed preparations
- (2) The investigator recultivating the molds starts with the actual spores contained in the original suspension
- (3) The space required for storage of a large number of lyophile preparations is much less than for any other type of culture (fig. 13 B)

Preservation in Soil

Soil has been successfully employed as a means of preserving vigorous stock cultures over long periods. As early as 1918 Barthel (Cent. Bakt. II 48:340-49, 1918) reported the successful maintenance of yeasts and bacteria in this medium and modifications of this technique are now employed in many laboratories for preserving bacteria. Greene and Fred in 1934 compared cultures of various molds preserved for two years in soil with the same strains continuously maintained on malt extract and malt extract-potato glucose agars and on bread. In their experience, soil preparations were most satisfactory and Professor Elizabeth McCoy (personal communication) has recently reported cultures of *A. sydowii* preserved in this manner to be viable after nine years. Since the publication of Greene and Fred's work, the soil method has been rather generally used by the Wisconsin group as a means of preserving valuable stock strains of molds. Furthermore, it is known to have been successfully employed during the past two years by a number of laboratories to maintain cultures of penicillin-producing molds in a high and uniform state of productivity. The soil substrate used by Greene and Fred was prepared as follows:

To air-dried orchard loam soil (Miami silt loam) sufficient water is added to bring it to a moisture content of about 20 percent. The soil is then transferred in convenient amounts (about 5 grams on a dry basis) to ordinary half-inch (1.27 cm) culture tubes. The tubes are plugged with cotton and given four 3-hour sterilizations at 15 pounds per square inch (1 kg. per sq. cm) pressure on alternate days and tested for sterility by addition of yeast water glucose broth to tubes selected at random. The tubes are then inoculated with 1 cc. of a heavy spore or mycelium suspension of the desired mold and kept at room temperature. That there is appreciable growth and sporulation on the soil can usually be ascertained without difficulty by direct microscopic observation. While the addition of nutrient to the soil may bring about somewhat greater growth, it does not seem to enhance the keeping qualities of the cultures.

It has been found possible to preserve on soil mold stocks used for large scale growth—namely *Aspergillus fischeri*, *A. sydowii* and *Penicillium chrysogenum*—for over 2 years without loss of their essential and desirable characters. Moreover, the gross colony characters have remained much more constant than did those of the corresponding cultures maintained in the usual way on agar slants. The soil cultures

can be recovered as required simply by streaking some of the soil particles on fresh agar slants.

It is not in all cases advisable to depend on soil alone for the preservation of valuable stocks but reserve stocks may without difficulty be prepared on soil and the writers believe that in many instances soil will be found to be an excellent medium for maintenance with a minimum of change over long periods of time.

During the past two years the soil method has been used at the Northern Regional Research Laboratory with but minor modifications of the technique cited above (fig. 13 C). Its principal advantages lie in the fact that (1) the viability of strains is apparently lengthened and (2) from a single stock tube opened with proper care repeated cultures can be started simply by removing some of the soil particles to suitable substrata.

Vegetable Substrata

The oriental fermentation industries maintained their inoculating material as selected rice or soybeans upon which the mold had been grown under favorable conditions to produce maximum quantities of spores. This nutrient dried and packaged was stored and sold under the Japanese name *Koji*. Samples examined after several years showed excellent viability.

Bainier was a pharmacist. He distributed licorice root in sections 5 to 10 mm in diameter and 5 to 8 cm in length in test tubes, sterilized them and kept his cultures regularly for years upon them. Tested by us the method was a very satisfactory laboratory practice. American mycologists have successfully used bean stems for the purpose. Apparently any organic material which provides frameworks of cellulose encasing sufficient nutrients to support mold growth without complete breakdown of the mass may be used.

CONTAMINATION

Mixed Strains

In the routine conduct of cultural work contaminations of cultures of one species of *Aspergillus* by other species or species of other genera is very common. The conidia of most molds are exceedingly light and are carried freely in the air. Entire exclusion of such contamination is difficult. In dealing with contaminations several problems arise and different procedures are possible. A colony of a single *Aspergillus* well established usually inhibits the growth of other species developing in the immediate vicinity. Even if invasion occurs the effects are commonly so distinct as to leave little doubt as to the limits of the different forms. When however the contamination with spores or mycelium is carried in the inoculum and so placed as to germinate in intimate contact with the organism desired (1) the species may sector out and hence be easily recognized or (2) the colony

Mites

Mites are very common in rotting vegetables, especially in partly dried condition in dried meat products, in hard cheeses, and in organic soil masses. They are thus common associates of molds as they occur in nature.



FIG. 15 *Penicillium* sp. parasitic on *Aspergillus niger* $\times 165$ (Photograph by Edward Yull)

hence the worker who handles moldy substrata in isolating his organisms must constantly watch for them. In size mites are commonly just about at the limit of visibility by the unaided eye. One accustomed to them will detect them readily but until seen and the appearance of their depredations

understood, they can pass unnoticed for considerable periods by persons who are otherwise good culture workers. Mites will crawl from petri dish to petri dish leaving behind them a trail of bacterial and mold contaminations as well as streams of eggs which develop rapidly into more mites that actually destroy the colonies. Since mites have preferences as to food some species are invaded and others avoided. To reach an attractive food supply a mite will frequently go through a cotton plug as ordinarily made and occasionally seems to get through even a paraffined plug. As a factor in the mixing of strains of molds in a laboratory collection mites must not be ignored.

Similar mixing and contamination occurs frequently whenever a laboratory becomes infected with ants, roaches or other insects.

Poisoning Cotton Plugs

Mites. Entire elimination of mites by sanitary measures is possible but often not attained. As a precaution in the preservation of stock cultures some scheme of poisoning should be used. One of these formulas consists of dipping the tips of the cotton plugs in a solution of the following composition:

95% alcohol	95 cc
Bichloride of mercury	0.5 gm
Glycerine	5 cc
Color with any aniline dye	

Care must be taken that the solution does not come in contact with the colony. The cultures must be allowed to develop into typical colonies before poisoning. In an antiseptic formula for the purpose needs alcohol to insure penetration of the plug, a poison to destroy the mites, glycerine to prevent the crystallization of the poison as the alcohol evaporates, and the dye to insure the destruction of the cotton plugs when removed from the tubes. In our own experience we have consistently made it a practice to wipe off the outside of all culture tubes with the above or some other sterilizing solution and to poison all plugs before cultures are replaced in or added to the collection of stock cultures.

Molds. Under humid laboratory conditions cotton plugs, especially if made from the absorbent type of cotton absorb moisture. Careless handling in preparation and care of such plugs often adds enough nutrients to support growth. Steam sterilization tends to distribute nutrients. Dirt, bacteria, and molds fall from the air upon the exposed portion of the plug. Handling detaches spores from the colony within so that both ends of the plug are commonly well seeded. Spore germination therefore may begin at either or both ends. Outside molds may grow through and drop into the

culture, or the culture itself may grow out through the plug and contaminate other cultures or experiments. Surface sterilization of the outside of the tubes and poisoning the plugs takes care of molds, as well as mites.

Spraying Only sprays, as selected fractions from petroleum, available from commercial sources, even kerosene distributed with a gun that produces a mist penetrating and filling all cracks, crevices, open spaces among apparatus or furniture and clouding the whole atmosphere of the laboratory, have been found effective in carrying down mold spores and bacteria from the air and ridding the laboratory of mites, insects, and vermin.

PRESERVATION OF DRIED SPECIMENS

Mold cultures lose many of their characteristic and diagnostic features upon being dried. Nevertheless dried herbarium specimens serve a useful purpose in preserving type material which might otherwise be lost. Details of morphology are often difficult to establish from such material, but group characteristics are preserved and over all colony appearances can be recognized after many years. The retention of culture tubes or petri dishes containing such dried specimens constitutes a reasonably satisfactory means of preservation, and the material contained therein approximates as nearly as is possible the cultural picture of the growing colony. Glass tubes and dishes however are cumbersome and easily broken hence may prove unsatisfactory if frequent handling is necessary. For many years we have employed an alternate technique with generally satisfactory results. Representative portions of colonies grown in petri dishes are cut with a large cork borer, lifted out with a spatula and dropped into paper pill boxes where they are allowed to dry. These can then be stored in larger boxes or attached to herbarium sheets for filing. The boxes should be provided with tight fitting lids, and for greatest convenience should measure approximately $1\frac{1}{2}$ inch in diameter. Aspergilli stored in this way prove useful in many comparative studies. They cannot however under any condition take the place of carefully handled living cultures.

CHAPTER VI

VARIATION

The *A. pergilli* are a variable and mutable group of fungi. They are characterized by great diversity and variability as they are isolated from nature and an increasing amount of evidence shows that they can be made to vary or mutate in the laboratory by subjecting them to a number of different imposed stimuli. Frequently the same types of mutants or variants ultimately result under both natural and artificial conditions. Nevertheless it is believed desirable to consider somewhat separately variations and mutations resulting from natural causes and those resulting from imposed stimuli.

Definition of Terms

Before entering upon a discussion of variation either natural or induced it is important to define certain terminology which is to be employed. It is recognized that our definitions will not agree in all cases with those of earlier workers nor do we expect that all subsequent investigators will accept those which we propose. If the meaning in the present discussion is clear our purpose will have been served.

(1) The term *mutant* or *mutation* is used to designate a strain whose source is actually known and can be verified. Furthermore it is limited to those substrains which originated as sharp breaks from parent cultures (usually interpreted as gene mutations) and in successive culture generations retain their distinguishing characteristics unaltered. This may or may not have a taxonomic connotation. Upon occasion it is used in essentially the same sense as *variety*. To illustrate *A. nidulans* mut. *albus*, *A. fumigatus* mut. *helvola*, *A. niger* mut. *cinnamomeus* etc. are used as Latin names to designate forms which differ from the parent species in certain striking details. In other instances it is used to identify a type of change rather than to designate a particular and isolated strain resulting from such change. It is considered correct to refer to artificially produced albino, yellow, and buff-colored strains of *A. terreus* as mutations (see p. 75) since they are constant in character and are known to have originated from a cinnamon-colored parent culture wholly representative of the species and we believe it represents good judgment to refrain from assigning Latin designations to each of them. The term *mutation* then refers to altered strains of constant character and known lineage whether or not they are given Latin designations.

(2) The term *variant* or *variation* is used loosely and reference to it in this manual is not, in all cases, entirely consistent. In general however it is applied to subcultures, or strains, arising through gradual change from well-defined strains or identifiable species. The characters of a variant, then, are not generally stable but subject to continued change and further variation. As used by us the term has no taxonomic implication and can be considered essentially synonymous with the term 'saltant' which is so commonly employed in reports on variation in the *Fungi Imperfecti*. Variants frequently appear as colony sectors, overgrowths, or other localized areas of changed appearance or texture. When isolated in pure culture they may or may not retain their distinguishing characteristics.

NATURAL VARIATION

Cosmopolitan species and groups of *Aspergilli* show adaptability to wide ranges of environmental conditions. As these molds are isolated from nature variation among the members of any species series or group is regularly encountered. Such variations commonly differ in degree rather than in basic characters and one can distinguish a series of intergrading or bridging forms. Even striking isolates are often unmistakably allied with some well defined species or group in this manner. Such different but intergrading forms arising in nature can be considered as natural variants. Natural variants of a similar kind can frequently be obtained in laboratory culture by selective isolation and cultivation from sectors or other areas of atypical growth, or by single spore isolations. Distinction must be drawn between differences in appearance morphology and habit of growth resulting from inherent differences between strains and alteration in colony character in response to changes in the composition of the culture medium or other environmental factors. Rigid comparative culture is often necessary to distinguish between the two. For the present discussion we are concerned with differences that are more fundamental than direct temporary responses to artificial stimuli (ecads) but the latter unless carefully evaluated may appear no less real. Rightly or wrongly Blochwitz (1930, p. 247) comments that *A. flavus*¹ in specimens collected in the Botanical Garden at Buitenzorg was called *A. penicillopsis* (Henn.) Rac. in the Botanical Garden at Singapore. *S. vitellina* Ridley in India. *S. corolligena* Massee and in Columbia *A. delacroixii* Saccardo.

Occasionally isolations are made from laboratory cultures which represent sharp breaks from the parent strain and since they are constant in subsequent culture they may be considered as true mutations. Representative

¹ The name of the original describer is only used for specimens or strains in culture which were definitely attributed to the describer. *A. flavus* *A. niger* *A. terreus* etc. are series concepts as used here.

of such natural mutations which originated in the absence of any artificially imposed stimuli are *Xantho* *A. fumigatus* var *helvola* (1939), *A. nidulans* var *albus* (1939) and *Cladosporium olivaceum* (1938)

Intra strain Variation

A certain amount of variation can be expected to occur in any given strain of *Aspergillus*. In certain species and strains this is very limited and cultures can be re-cultivated repeatedly upon a variety of media and at different temperatures and H ion concentrations without evidences of visible change other than those resulting from the immediate effects of the altered environment. Many strains of *Aspergillus niger* are characterized by such comparative stability. Other species and strains are subject to continual variation with differences in character and rate of growth appearing rather abruptly as sectors or overgrowths or gradually developing as a progressive alteration in the general aspect of the whole culture. By successive and selective subculturing strains of *A. alliaceus* and *A. ochraceus* showing a marked difference in sclerotium production can be obtained. In like manner strains of *A. staccinicus* Kunoshita can be secured which are almost completely sterile upon all media tested. The same is true of *A. granulatus* Raper and Thom. *A. flavipes* etc.

Working with a strain of the ascomycete species *A. fischeri* Greene (1933) isolated 448 single spore cultures and among these found variant progeny of two main types: (1) cultures producing very large scattered perithecia as opposed to the typical picture of many small perithecia and (2) cultures producing conidial structures in profusion but forming few perithecia and these tardily. The second type was fairly stable whether derived from single ascospores or conidia. The first type was variable in some cases reproducing the characters of the variant parent in others reverting to the character of the original stock culture.

Hansen (1938) and Hansen and Smith (1932) have studied many of the Fungi Imperfecti rather exhaustively and report that single strains of these fungi are basically composed of a mycelial (M) type and a conidial (C) type. By proper techniques the two forms can be separated and recombined at will. While it has not been explored as yet the possibility exists that the same condition may prevail to a limited degree in the Aspergilli and that this may account for a certain amount of the intra-strain variation encountered.

Back of variability in molds many lines of discussion have been developed. Buller (1933) has frequently called attention to the unmeasured possibilities of nuclear and cytoplasmic disturbance from the commonly observed phenomenon of anastomosis. Vegetative hyphae belonging to the same mycelium (mycelium derived from a single spore) or different mycelia

throw out branches which fuse without showing any other sign which might suggest a sexual process either before or after fusion. Anastomosis is usually observable, if at all, in the rapidly growing area where many spores placed as an inoculum are developing into one colony. By transferring large numbers of spores from an old culture to a new one, most of the *Aspergilli* studied by us have shown fairly consistent repetition of colony characters and conidial morphology and have been maintained for a long time with little or no observable change. Other organisms handled in the same manner have not been successfully maintained with the morphology originally studied. Differences in behavior can be attributed to variability between strains.

Intra species Variation

Variation within the species is very prevalent and is well marked in many cases. When a large number of isolates of any particular species or series is collected, one can regularly expect to find among them wide variations in color, amount of sporulation, and in their general habit of growth. Usually, however, such variation is graduated and strains representing various intermediate steps between the extremes are to be expected. While it is by no means unique, we may use *A. terreus* as an example since a very large number of strains belonging to this species have been isolated and observed in plate and tube cultures during the past two years (fig. 16). Colonies of the type strain and of the great majority of isolations made from nature, are plane, cinnamon in color, very heavy sporing with conidial heads arising directly from the substratum in an even and close stand. An occasional isolate is much brighter in color approximating xanthine orange (Ridgway Pl. III) but in all other respects it is fairly typical. It is believed to represent a form such as that described by Blochwitz (1934) as *A. boedyni* and in the present manual we have designated it as *A. terreus* var *boedyni*. In each of the above cases, the production of abundant fruiting structures follows closely the advancing margin of the growing colony and there is little or no continued growth of mycelium except in the marginal area. Certain other strains are quite floccose; conidial heads are typical in form and color but are greatly reduced in number and are borne upon aerial hyphae. Shih (1936) was probably working with such a culture when he described the variety *A. terreus* var *floccosus*. We feel that the forms are sufficiently distinct to warrant maintaining his variety. Still other strains possess abundant but fairly close- rather than loose-textured mycelia and bear abundant but very pale buff-colored conidial heads. The vegetative mycelium in this form is bright yellow and for this reason we have designated it as *A. terreus* var *aureus* n. var. (see p. 198). If one should examine only the type strain and these three atypical forms it is entirely probable that one

would describe them as four distinct species. Actually, however they are not sufficiently distinct to warrant specific rank for they represent only extremes of variations along three divergent lines with numerous inter

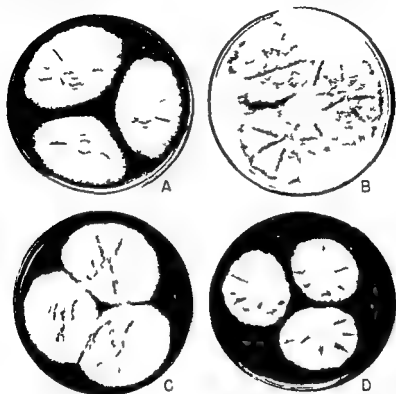


FIG. 18. Intraspecific variation in *Aspergillus terreus*. A *A. terreus* typical strain NRRL No. 765 characterized by heavy conidium production and colonies cinnamon in color. B *A. terreus* var. *boedijnii* NRRL No. 680 characterized by deeper colonies and conidial heads near xanthine yellow. C *A. terreus* var. *floccosus* NRRL No. 1921 characterized by loose floccose colonies and spore heads light pinkish cinnamon in color. D *A. terreus* var. *aureus* NRRL No. 1923 characterized by yellow floccose colonies and comparatively few cream to buff-colored conidial heads.

grading strains aligning them almost without interruption with the typical form itself.

Another series of variants in our collection shows gradation from the usual radiate head and conidiophore of *A. sydowii* to the simple mono verticillate penicilli of *Penicillium restrictum* Abbott. The tendency to form

reduced conidial apparatus is observed in all of the strains of *A. sydowii* examined. Over a period of many years variants have exhibited all gradations in colony appearance from typical *A. sydowii* to the aspect of *P. restrictum* of Abbott except for the presence of an occasional conidiophore and head of *A. sydowii*. Repeated cultural tests exclude contamination. These variants occur in nature, they produce abundant conidia, and they undoubtedly maintain themselves successfully in the field.

Aspergillus fumigatus presents a somewhat similar condition. In this species, typical cultures produce heavily sporing, velvety colonies that are dark green in color and show almost no aerial mycelium. Other strains commonly isolated from nature produce very floccose colonies and bear comparatively few conidial heads (fig. 37). These heads, however, are typical in form and in dimension. Strains possessing this contrasting character are relatively stable in culture but in this species as in *A. terreus*, all degrees of intergradation are found between this floccose type and strains entirely typical of the species. The same story is repeated in other species.

Appreciable variation can normally be expected among the isolates of any of the very abundant and cosmopolitan species. Such variant strains, however, are the exception rather than the rule since the great majority of isolates are quite typical of the species. Attention was called to this fact in our study of the *A. glaucus* group (1941).

When grown in comparative culture strains successively isolated as representing a particular species usually show enough difference to give each strain a kind of individuality. Exact identity point by point is not expected. Such strain variation may be incidental and unimportant or it may be correlated with activities which make one strain a valuable agent in an industrial process and the other worthless.

Intra group variation

Inside the different groups of *Aspergilli* one normally finds somewhat similar but wider variations than those seen among the strains constituting any particular species. To what degree species in nature have developed by mutations and by progressive variation can only be guessed. We do know, however, that the species within a group like the strains or varieties within the species are regularly bridged by intermediate forms which render it difficult to establish sharp and immutable lines of separation. One can almost cite it as a rule that the definiteness with which one regards a species is inversely proportional to the number of strains of that species which have been examined. Still species are necessary as guide-posts—as fixed points around which closely related organisms showing a certain but limited amount of variation can be grouped.

The *Aspergillus flavus oryzae* group can be taken as illustrative of the type of variation to be expected within a group of the *Aspergilli*. Thomas

culture No 113 (NRRL No 447) of *A. oryzae*, received from Baarn and believed to stem from Cohn's original strain is a very floccose loose-textured culture bearing comparatively few small light yellow to tan heads. Conidiophores are long ranging up to 2 to 5 mm and are very thin walled. There is only a trace of green even in individual heads and in general aspect the culture normally shows no green color. *Aspergillus flavus* in its typical form is not floccose and is very heavy sporing. Conidiophores arise directly from the substratum in a close stand, are usually 1 mm or less in length and are comparatively heavy walled. Colonies are regularly in yellow green shades and range from light to comparatively dark green (see species description). *A. parasiticus* Speare goes even farther. Colonies are very dark green in color. Conidiophores usually range from 200 to 400 μ in length and sterigmata are typically in a single series whereas they may be in a single or double series in *A. oryzae* and *A. flavus*. In the opposite direction but markedly different from *A. oryzae* is *A. effusus* Tiraboschi. Typically this is very floccose and comparatively light sporing with heads borne upon short conidiophores which arise from the loose aerial mycelium rather than from submerged mycelium in the substratum.

The cultural pictures of these species are fairly characteristic. Yet it is practically impossible to take a large collection of 100 or more strains and separate them into these species with any degree of confidence or satisfaction. The difference between *A. oryzae* and *A. flavus* is bridged completely by a series of intermediate forms showing all degrees of variation between the two strains elected as typical. Nomenclature in this group is then further complicated by the fact that among the great collections of these forms obtained from the Orient and designated *A. oryzae* the majority of forms are somewhat intermediate between *A. oryzae* and *A. flavus* as depicted above. A similar series of intermediate forms bridges completely the gap between *A. flavus* and *A. parasiticus*. There is no sharp line of demarcation between any of these species still they are not one and the same and to attempt to lump these diverse forms together into one species as Neill (1939) has done for the *A. glaucus* group intensifies rather than reduces the difficulties encountered.

Intra-group variation is also particularly marked in the *A. niger* group. During an extended period of study and observation of molds in culture Biourge who was a discriminating collector accumulated 63 strains of black *Aspergilli* which suggested sufficient individuality to be deemed worthy of further study. These were turned over to Moneray when he entered Biourge's laboratory. He assumed that he had before him all of the black *Aspergilli* possible to collect and knowing that Biourge had selected each of them because it seemed to have some special character he undertook a taxonomic study to define those characters and to organize them into a systematic presentation (1934a). His paper lists 35 species of

which 25 were either described as new species or new combinations. His findings in *A. niger* are fairly illustrative of the same type of study in other groups (compare Thom and Currie [1916] for *A. niger*, Thom and Church [1921] for *A. flavus*).

Mosseray based his primary separations upon conidial sizes, shapes and markings, while secondary and tertiary separations were based upon conidiophore lengths and the characters of colonies in tube cultures on Biourge's "Raulin neutre gelose"—a variation of the classic Raulin solution. Biourge and Simonart demonstrated the entire series to one of us (C. T.) showing how wrinkling and granulation of mycelium, intensity and changes of secreted color, shades of color in the conidial area, lengths and proportions of conidiophores and heads, and their distribution over the mycelium gave to each strain an individuality which had been repeated in successive cultures over a considerable time. We raised just one question, 'What would you do with the next thousand?'

The large majority of all isolations of black *Aspergilli* conform within a range of minor variation with the general van Tieghem concept,—i.e. black brown colonies with conidiophores and heads giving the general structure and measurement of parts found in the classical description. Then, in contrast with these, there are shades of colony color from the coal black of *A. carbonarius* through shades of purplish black to the brown of *A. ferrugineus* Fuckel or to the lighter shades of Schiemann's mutants (pp. 223-224). Some of them produce no colors in the substratum and reverse of the colony; some show yellow in traces; others are persistently deep orange, giving the whole a yellowish appearance. Or, again, the agar and mycelium may develop a red brown or mauve shade of violet.

Conidiophores in the usual type of culture reach nearly enough the same length to give the effect of a field of grain. But their length may be quite short and the heads seem to be borne directly on the substratum, or they may be several millimeters in length with the heads borne well above the substratum and correspondingly large. Between these extremes every variation can be found. Conidiophores may be scattered thinly over the vegetative mycelium, collected in a zone at the border or crowded in the center.

The vegetative mycelium may grow as a flat felt (plane) or may be variously wrinkled, sulcate or buckled. In a smooth or plane colony the mycelial cells seem to stop growing early—the colony extends only at the margin. In the plane colony intercalary growth (i.e. the formation of new cells in the filament or the lengthening of the old cells), and the production of new branching ceases. Such mycelia ordinarily produce one crop of conidial heads beginning at the center of the colony and progressively developing toward the margin until the medium is exhausted or some inhibiting factor paralyzes growth. Marginal growth in such a colony

often shows longer and fewer conidiophores and larger heads than in the central area

Within the group with the usual structures still recognizable many variants with contrasting features appear. Heads with very long primary sterigmata which are sometimes septate appear in *A. carbonarius* (Bainier) Thom. *A. pulchella* Speggazzini or *A. turingensis* Mosseray. The primary sterigmata may grow out into sterile filaments as in Mosseray's figure for *A. ficuum* (Reich) Henn. much more commonly some primary sterigmata grow out as tiny conidiophores and produce little heads often consisting only of a cluster of simple sterigmata and conidial chains. Thus sterigmatic changes may run from the simple sterigmata of *A. japonicus* Saito *A. luchuensis* Inui or *A. maliacis* Mosseray where only some are double to other species showing the widest range in length and arrangement.

Another group of variants show marked suppression of the ordinary structures expected. Strains in which conidiophore formation has been reduced or almost suppressed have been studied in continuous culture. Such colonies showed an occasional long conidiophore and large head conforming to the *A. niger* pattern produced at the end of the colony growth period. Meanwhile the mycelium was fully covered with irregularly branching hyphal elements bearing single sterigmata variously placed groups of sterigmata or penicilloid clusters of sterigmata each bearing a short chain of conidia showing the characteristic markings of the group. Transfers from the simplest form developed the complex or *A. niger* elements. Transfers from the large heads brought a recurrence of the reduced type of fruiting. No method of selection tried brought back the typical *A. niger* aspect and cultures of this type appear to represent degenerate forms.

It would appear then that a general type or morphological picture when found dominant in large numbers of natural isolates can be regarded as typical for a species of *Aspergillus*. It is recognized that marked divergences from such types occur under the unrecorded stimuli of nature. Some of these forms succeed in establishing themselves as permanent elements of the microflora and thus become successful as species or varieties. Others do not digress quite so markedly and thus constitute intermediate or bridging forms. At the same time marked changes can be induced by the application of artificial stimuli. Where the origin of such altered strains is known they are commonly regarded as mutants. Were they isolated directly from nature and their previous history not known it is probable that they would be considered as separate varieties or even species.

Natural Mutation

While most of the mutants which we recognize as such have originated in the laboratory as the result of certain artificially imposed stimuli (or

drastically altered conditions of growth), and hence can properly be termed induced mutations a number of well authenticated cases of natural mutation are known In 1939 Edward Yull described as *A. fumigatus* var *heliola* a buff colored mutant of this species isolated by him in 1937 (fig

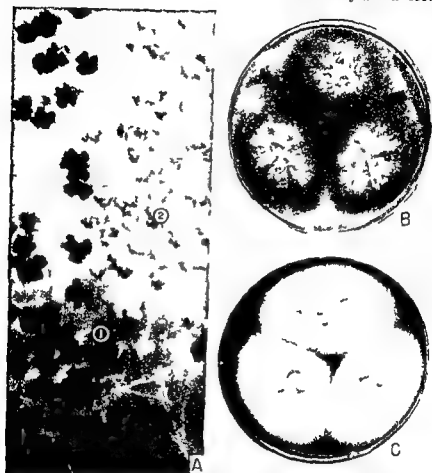


FIG 17 Natural mutations 1 Portion of a colony of *Aspergillus niger* in which a tan spored mutation appeared as a V sector A₁ typical black head of parent strain, A₂ tan head of naturally occurring mutation X 75 approximately B and C Typical strain of *Aspergillus fumigatus* and a naturally occurring mutation discovered and described by Edward Yull as *Aspergillus fumigatus* mut *heliola* The mutations in both species have proved completely stable in continued culture

17 C) In the same report a white-spored mutant of *A. nidulans* isolated in 1937 was described as *A. nidulans* mut *alba* In both cases the mutants developed as natural phenomena without the application of any artificial stimuli—in the former case as a single head in the later case as a group of heads and in both cases from wholly typical strains (fig 17)

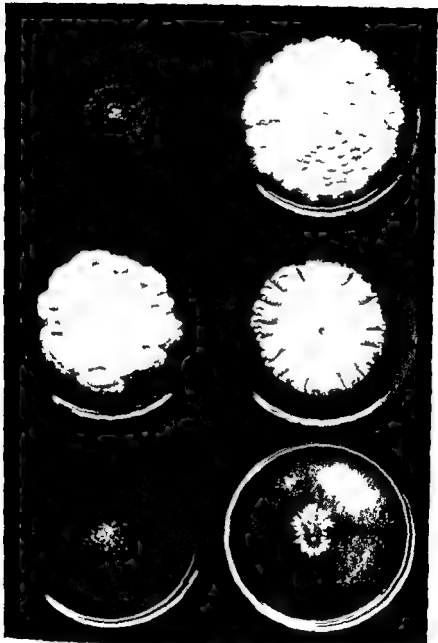


Fig. 11

M. lat. w. 4 pe. gill. free. prod. cell hy. rad. t. g. c. p. 1. 0. ltra. 1. t. l. t. C. l. ma. row.
 C. k. ad. to. an. t. roo. to. perat. f. t. eek. 4. t. the left. 4. pe. l. t. N. B. R. L.
 N. 295. rad. t. l. took. l. re. B. l. 1. per. g. l. t. All. t. t. prod. g. l. to. co. d. l. l. ad. h. t.
 m. g. l. h. ad. l. re. rpl. h. g. cal. l. rae. t. r. n. l. t. pare. t. l. re. t. re. t. le. t. floose.
 3. l. w. l. to. t. t. prod. g. l. w. 3. t. t. cal. l. l. l. ad. D. (re. t. right) Le. t. r. y. t. ant. pro. l. g.
 l. m. to. t. re. l. e. k. e. n. l. ry. f. w. 3. t. p. cal. l. l. l. p. l. left. N. t. r. t. ut. g.
 f. m. hall. 6. no. t. r. o. t. t. r. o. d. t. l. p. d. g. c. l. ea. l. f. b. t. rely. t. y. cal. l. p. l.
 f. t. g. l. t. Tl. m. t. t. f. l. l. to. p. l. ce. t. prod. M. y. r. o. s. i. g. c. o. u. o. ad.
 y. few. d. t. y. p. cal. l. d. l. l. ea. l. sea. t. e. t. p. 5. C. l. o. l. t. t. right. l. l. new. North. m. R. g. 1. R.
 March Laboratory. R. prod. ced. t. l. no. gh. p. o. o. e. t. f. C. l. o. l. h. d. Co. l. p.

In studying a group of cultures three years ago, the authors noted a few tan heads in the form of a V-sector in an otherwise typical black colony of *A. niger* (fig 17 V). Isolations from a tan head reproduced the mutant head characters and repeated transfers of this strain have proved consistently stable over a period of three years. The mutant strain cannot be distinguished from *A. niger* mut. *cinnamomeus* (*A. cinnamomeus* of Schlie-mann).

In dealing with natural as well as induced mutations we have endeavored to limit the use of the term mutant to forms whose origin was definitely known. Blochwitz (1934, 1935) was not so precise. Under the name *A. glaucus* mut. *alba* he refers to a white-spored member of the *A. glaucus* group. He believed *Aspergillus giganteus* Wehmer to represent essentially a long-stalked *A. clavatus* hence designated it *A. clavatus* mut. *giganteus*. This treatment may be justifiable. We believe however that until their origin from other and well marked species can be proved it is wise to continue to recognize as species these very distinct forms that are isolated from and are able to maintain themselves in nature.

Cladosarum. The most striking variant or mutant ever described in the *Aspergilli* is *Cladosarum olivaceum* of Vuill and Vuill (1938). This appeared in a culture of *A. niger* growing on bread at 28° C. (Personal correspondence). Its colony conidiophores vesicles and primary sterigmata are those of *Aspergillus*. The secondary sterigmata instead of producing conidia thrust out cells which are essentially the same in morphology as the secondary sterigmata themselves. The same procedure is then repeated several times. Occasionally however a terminal cell changes and thrusts out several equal cells. In other words it resumes the function of a primary sterigma. The new secondaries repeat the process of producing chains of cells each resembling the basal cell with the aspect of a sterigma not a conidium and always with the youngest cell at the tip of the chain. In *Aspergillus* the sterigma which produces a chain of conidia always produces the new conidium at the base of the chain shoving the next most recent farther out. Differing then from Vuill's interpretation *Cladosarum* produces no conidia however readily any cell detached from the mass may grow.

In *Aspergillus* the ordinary nuclear procedure in conidium formation involves mitosis in the sterigma actually producing the conidia. After each mitosis one daughter nucleus migrates through the tube into the new spore in which it rests until that spore begins to germinate. The other nucleus remains in the sterigma and repeats the process. This goes on until there may be a chain of 200 conidia—the oldest at the outer end the newest directly attached to the sterigma.

In the absence of cytological study one may offer the following hypothesis. In *Cladosarum* the nuclear procedure must be reversed

The same mitosis occurs. One active and one resting nucleus result, but the resting nucleus remains in the sterigma while the active nucleus moves into the newly forming cell. This determines the course of development. The active, multiplying nucleus is always in the newest cell formed.

Previously Barnes (1928) had stimulated a strain of the *A. glaucus* group (identified by us as *A. amstelodami*, 1941) by heat, and reported certain mutants which were deposited with Dr. Westerdijk at Baarn. Among them, under the designation "Creamy" (NRRL No. 143), a mold with the morphology of *Cladosarum* appeared. It was obviously derived from some *A. glaucus* strain and is, in so far as the writers are aware, the only other appearance of the *Cladosarum* structure ever discovered. Barnes does not appear to have recognized its contrasting structure.

Since no collector has reported this type of mutant in nature, it must either be very rare or be unable to maintain itself in a competitive environment. However readily such mutants may be maintained in the laboratory, they would rarely reach the second generation in nature on account of lack of spores. The name *Cladosarum* was thus applied to a defective organism (zoologically designated a "monster"), which does not become a component of any natural flora; hence taxonomically the name should be untenable.

INDUCED VARIATION

Striking mutations have been obtained from various species of the Aspergilli by subjecting them to artificially imposed stimuli. Schiemann (1912) was among the first to draw attention to the possibilities inherent in this approach. By subjecting a strain of *A. niger* to various concentrations of potassium bichromate she was able to produce two striking mutations which she designated according to color: *A. fuscus* (= *A. niger* mut. *schiemanni* of this manual) and *A. cinnamomeus* (= *A. niger* mut. *cinnamomeus* *ibid.*), respectively. Both cultures have remained stable in our hands and in various collections throughout the 32 years since their original isolation. A third "mutation" designated *A. niger* var. *altipes* could not be distinguished from other strains of black Aspergilli isolated from nature—the parent strain was not seen. Working with a member of the *A. glaucus* group designated *Eurotium herbariorum* Wigg. Barnes in 1928 reported the production of a series of variations by exposing spores to heat. While there are reasons for questioning the correctness of some of Barnes' interpretations and conclusions (see Thom and Raper 1941) there is evidence that he succeeded in producing a mutant which in its habit of growth and in the character of the fruiting structures developed bears a striking resemblance to a form subsequently isolated from *A. niger* by the Vuils (1938) and described by them as *Cladosarum olivaceum* genus and species.

new Galloway (1933) obtained marked variation in colonies of *Aspergillus terreus* by growing them upon media containing flour to which was added 0.003 to 0.005 per cent of saheylandide

Thom and Steinberg (1939) and Steinberg and Thom (1940a, 1940b) in a series of experiments applied chemical stimulants to a strain of *A. niger* (Thom No 4247 NRRI No 331) which had been in the collection many years without noticeable change. From the cultures resulting Steinberg picked out and purified for study all variants he could observe with the naked eye and with the aid of a hand lens. In examining a fruiting area of a colony general changes were not common; ordinarily an occasional head changed color, long or short conidiophores appeared in spots, gross malformation showed as areas of no fruit or too much fruit or color effects in the mycelium. These were picked out and grown in successive cultures. Expressed in terms of morphology the most striking feature of the tested culture was disturbance of uniformity. The same types of changed aspect were reproduced many times. In general they followed the same lines as have been described as present in the natural series selected by Biourge or in the collections of Mosseray at Brussels. In general these changes were destructive in character and included large numbers of injury mutants or variants which reverted in subsequent transfer to the original aspect of Steinberg's culture. Sodium nitrite was the most effective agent used. Some isolations however represented clean cut mutations. Strains of *A. fumigatus* with albino heads were obtained. Forms of *A. niger* with light brown to cinnamon-colored spore heads essentially like those earlier obtained by Schiemann (1912) those obtained by Whelden (1940), and those subsequently obtained by Raper, Coghill and Hollander (see below) from members of the same group were likewise isolated. These have remained stable in culture for the four years that they have been in our collection.

Whelden (1940) succeeded in obtaining a series of mutants in *A. niger* by bombarding conidia with low voltage cathode rays and subsequently isolating colonies which developed from such irradiated cells. Forms possessing heads in various brown shades rather than black were isolated as well as one giant form with conidial structures appreciably larger than the parent. By means of ultra violet irradiation of spores Raper, Coghill, and Hollander (in press) obtained mutants which produced tan-colored conidial heads but otherwise closely resembled the parent strain. In a more exhaustive study of *A. terreus* (Pl. II A) the same investigators succeeded in isolating a number of striking and markedly different mutations. These included albino forms with colorless conidial heads (Pl. II B) forms with pale buff-colored heads, yellow, yellow-white floccose forms with few and smaller conidial heads (Pl. II C) forms producing very thin sparsely

sporulating colonies forms producing restricted colonies characterized by the production of an excessive amount of orange brown exudate, and forms with leathery, close textured colonies bearing very few conidial heads (Pl II, D) Alterations in microscopic details commonly accompanied these changes in colony appearance (fig 19) In addition to the morphological mutants, which were found to be stable when checked through ten successive transfers over a period of 12 months, various physiological mutants were also isolated These included forms unable to utilize nitrate nitrogen (Pl II, E) but able to grow and sporulate normally upon media containing ammonia nitrogen and a form unable to synthesize thiamin (Pl II F) Upon Czapek's solution agar containing sodium nitrate and sucrose, each of these is strikingly different from the parent strain upon

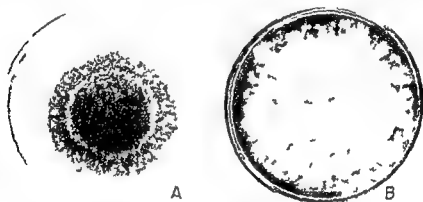


FIG 18 Induced mutation A and B *Aspergillus niger* group strain NRRL No 67 A parent culture growing on Czapek's solution agar 10 days room temperature B tan spored mutation of same produced by ultraviolet radiation

malt extract agar which contains adequate amino nitrogen and thiamin neither could be differentiated from the parent (Raper Coghill and Hol laender in press) Thus the need for comparative study and examination upon a variety of media is apparent while the importance of such studies in reliable taxonomic work cannot be over-emphasized In cultures of *Aspergillus terreus* resulting from irradiated spores the capacity to produce itaconic acid varied from zero in some isolations to levels somewhat above the parent culture in others The majority of such isolations produced yields somewhat lower than the parent strain many produced yields approximately equal to it while a very few produced superior yields (Lock wood Raper Moyer and Coghill in press)

Based upon our own investigations and the published reports of other

workers certain observations of a summary character can be made regarding variation in the *Aspergilli*:

1. *Aspergilli* include strains and species adapted to a very wide range of environmental conditions. Such conditions may influence materially the cultural and morphological characteristics of these molds.

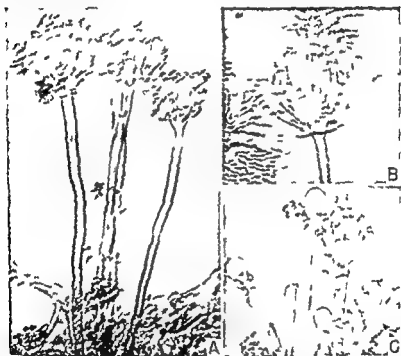


FIG. 19. Photomicrographs showing details of structure in the conidial heads of the parent strain and in two selected mutations of *Aspergillus terreus* (NRRL No. 265) produced by ultra violet radiation $\times 600$. A. Typical heads of non irradiated parent strain. B. Mutation in which conidium formation is incomplete and cells adhere in long chains in liquid mounts. C. Mutation in which many fruiting structures develop vesicles but often fail to produce sterigmata and spores.

2. Under natural conditions great numbers of variants appear along with occasional sharply separable forms or mutants which are definitely of species rank. The extent to which natural mutations may account for described species is a matter of conjecture.

3. The *Aspergilli* can be made to mutate in the laboratory by subjecting them to a variety of different excitants or stimuli. Induced mutations may parallel some of those found in nature and described as species.

4 Particular species of the *Aspergilli* tend to mutate along certain definite lines, e.g., the production in *A. niger* of forms with tan to light brown spore heads (Schiemann 1912 Steinberg and Thom, 1940, Whelden 1940, and Raper, Coghill, and Hollaender, in press), and the production in *A. fumigatus* of forms with colorless spore heads (Yull 1939, and Steinberg and Thom, 1940a). The type of mutant produced is not governed by the type of treatment given, although the number of mutations produced is strongly influenced by this factor.

5 Great variability in biochemical activity is encountered among strains isolated from nature, but these differences are rarely linked with specific morphological changes.

6 The *Aspergilli* can be made to mutate physiologically as well as morphologically by the application of various stimuli. Physiological mutations may conceivably be of tremendous importance in the development of improved strains for fermentation processes.

7 Despite natural variation, most strains of *Aspergillus* when subjected to critical transfer and maintenance under rigorous culture conditions can be kept for many years with constant colony appearance, stable morphology, and dependable biochemical activity.

PART II

THE MANUAL PROPER

CHAPTER VII

THE USE OF THE MANUAL

Since the purpose of this manual is to facilitate the identification of *Aspergilli* as they are isolated from nature and as they are encountered in the investigation of special problems the procedures and considerations involved in the use of the manual must be discussed. The general morphology and structural details found in the spore-producing apparatus of the *Aspergilli* have been described and figured in Chapter III. Complexities in the specific combinations of these characters found in the examination of moldy material and in the isolated colonies of individual strains makes desirable a summary outline of the exact observations to be made in describing an *Aspergillus*. Such a descriptive sheet is presented as page 82. For practical use a standard sheet of record paper is folded over on the left hand margin for about 5 cm. The column of observations desired is written upon this marginal fold; a fresh sheet of paper is slipped under the fold and the descriptive data are filled in, appearing exactly in the same order for each strain studied. A single glance at the sheet shows the discrepancies, if any, in the descriptive data obtained. With such a sheet properly filled out the keys to groups and within groups facilitate the placement of the strain in its proper group first, then its allocation to species within the group.

Such descriptive sheets to have comparative value in species diagnoses must present their data in standardized terms. It has therefore been necessary to define and illustrate the morphological terms accepted in this manual and to indicate as synonyms in the chapters on morphology the usages of various describers of *Aspergilli* back over the 200 years since Micheli.

Identification from specimens. The field mycologist working with specimens collected and examined fresh or dried will often find completion of a technical description very difficult and some observations impossible. One with long acquaintance with the *Aspergilli* may place his specimen to the group or aggregate species correctly but even such workers are frequently puzzled. If the organism is deemed important the fresh or recently collected specimen should be taken to the culture laboratory to insure its isolation and preservation in pure form. The descriptive data at hand should then be checked and supplemented from the pure culture.

To identify an unknown *Aspergillus* the worker needs pertinent data which will permit him to interpret his mold in terms of species already de-

scribed—including the observations essential in a species character. For convenience such data may be indicated vertically upon a descriptive sheet which is elaborate enough to include observations that are equally as useful. Measurements should be presented as ranges encountered in the examination of many units, *not as exact and single measurements of individual cells or structures*

Aspergillus—identifying number or marks	Primary sterigmata
Culture medium or natural substratum	measurements
Temperature of incubation	arrangement
Colony characters	color
Rate of growth	Secondary sterigmata
Texture	measurements
Mycelium	Conidia
submerged	color
floccose	measurements
color above	markings
reverse	Perithecia
Heads	color
color	size
form	shape
measurements	Ascospore
Conidiophore	color
length	size
diameter	markings
wall thickness	Sclerotia
markings	color
color	size
Vesicle	
shape	
size	
color	

With such a descriptive sheet before him the user of the manual finds that the Aspergilli have been arranged into a series of natural groups (20) each containing one to several species aggregates. Each group includes species with varieties and at times mutants having a series of essential characters in common. These groups have been arranged as near as possible in natural order, based upon the presence or absence of certain contrasting intergroup characters.

These major separating characters are usually evident and positive. Nevertheless individual species are found in which certain of these characters are reduced to vestigial or apparently suppressed, yet which show many characters allying them with a particular group that such placement is more logical than any other. Such species must sometimes be arbitrarily placed and their possible affiliation with other groups indicated both in the discussion of the species and in the discussion of the related group.

Species

The species concept in *Aspergillus* is very difficult to define in tangible terms. In this manual the species names already in use have been preserved wherever possible. The actual material originally described under a particular species name (i.e. type material) exists for but a few species. If such material exists, it is more important as fixing one point, one individual strain in a series of intimately related variants than tying the name to extremely definite morphology. If such material is not known comparison of large numbers of strains in pure culture with authoritative descriptive information supplemented by laboratory usages coming down from the original describer usually fixes the series or form intended.

From such composite sources it is commonly possible to establish a fairly concrete morphological aspect based upon ranges of color differences in structure, and variations in spore measurement which are repeated in great numbers of isolates. In such series of isolates there are no sharp lines of demarcation when large numbers of strains are brought together. Within our concept a single strain may show much of this variability within its colonies in culture or it may reduce or suppress certain characteristics and intensify others. Such variants have often been given species rank by workers unaware of the existence of other variants completely bridging the gap between such forms and other members of the series. Great differences in biochemical activity may be shown by different strains with or without contrasting morphology. Nomenclature based upon an assumed correlation of a particular cultural aspect with industrial significance has been offered but has proved utterly unreliable in identifying an organism if lost or in seeking a new strain to serve the same purpose.

Two contrasting tendencies in classification are always encountered. In the *Aspergilli* these may be represented by Mosseray (1934a) who found diagnostic marks to distinguish 35 species among 63 cultures of black *Aspergilli* in the collection of Biourge at Louvain. He later received many more variants and faced the question whether to try to describe them all or abandon the field. He admitted inability to write descriptions explicit enough to identify them all. In contrast Neill (1939) disregarding ascospore measurements and markings 'lumped' all of the *A. glaucus* group into *A. glaucus* Link. Likewise all of the black *Aspergilli* were considered as *A. niger* van Tieghem. Forms that he did not happen to recognize as belonging to one of his groups were discarded. These are extremes.

With abundant living material before him the student of the *Aspergilli* can usually recognize as representative a reasonable number of forms which can be described in tangible specific terms. Commonly forms which actually play a significant role in nature or in biochemical processes can be selected as the points around which such species descriptions are drawn.

On the other hand forms such as *A. janus*, *A. itaconicus*, *A. lutescens* etc., while probably rare in nature, possess sufficiently distinctive morphology to warrant species recognition irrespective of other considerations.

Varieties

The taxonomic term, variety is used here to designate any homogeneous member of a species complex which carries most of the diagnostic characters of the species but maintains one or more clearly defined differences in particular characters. For example variety *alba* is used for certain strains of particular species in which the characteristic color of that species is absent.

There is little agreement in the literature in the application of the term variety, certain authors use the term to indicate their belief that one form with particular morphological characters had its origin from another. In such cases the belief is hypothetical not a matter of observation. Sometimes previously known species were merely moved to varietal standing without specifying the characters upon which the decision was based. Such changes are reduced to synonymy or, if entirely unsupported, are occasionally ignored in this manual. The term variety is only useful if definitely associated with a clearly defined variation in structures within an otherwise homogeneous series of strains.

Mutations, or Mutants

The term mutation, or mutant is only recognized here for forms resulting from a sharp break in morphology (including color) from known structures characteristic of a species, to a definitely altered and inherited contrasting structure. Obviously the only excuse for the term in taxonomic usage is to designate the origin of the form studied. If the source of such a variant were unknown the taxonomist would designate the form present as a variety or species depending upon the nature and importance of the changes encountered. The increasing number of studies in experimental evolution make recognition of induced variation taxonomically necessary.

New Species

The discriminating collector will occasionally find an organism markedly divergent in characters from any described form. Usually the divergences leave the organism readily recognized as a member of one of the great groups. If the differences in aspect and detail of structure separate such a form from the other described members of the group and if the form is found often enough to prove that it has a place in nature description as a new species is warranted. Similarly an occasional form either by sup-

pression or complete disappearance loses the arbitrary diagnostic character which furnishes the basis for separating two adjacent groups. In such cases it has at times seemed more practical to add such a species to the group most nearly allied to it by general colony aspect but to cross-reference it to the related group.

The detection and description of species hitherto unrecognized necessitates extensive review of the literature and re-study of available living cultures of at least one whole section of the great genus *A. pergillus*. Unless the one who encounters a form that he cannot recognize under names already in the literature is prepared to investigate his form adequately in relation to the whole genus he should not describe his organism as a new species.

Summary ed

Comparative study of the taxonomic literature brings out the need for a standardized series of morphological and descriptive terms into which the many usages introduced in the two centuries since Micheli can be translated. Variations in measurement are deemed significant only if they exceed the common limits between closely related organisms and predominate in the preparations examined. Ranges in measurements are more significant than exact dimensions of either selected structures or averaged values based upon many measurements.

Merely quantitative variations are not recognized as warranting separation of species. For example differences in the shade of color or the intensity of a particular reaction especially when other strains are found to fall between the old and proposed new species are not regarded as species characters. Such proposed names either fall to varietal status or to synonymy.

The names not accepted here fall into several categories. (1) Many are listed as synonyms because they are believed to have been given to variants not recognizable by dependable and interpretable differences from other members of the same series. (2) Fantastic variants or monsters appearing in culture may like *Cladosarum* be maintained in the laboratory but unless found perpetuating themselves in nature clearly fall in the class of nature's experiments which do not contribute to the permanent flora. Such names are not regarded as established. (3) Unidentifiable species—names appearing in the literature based upon structures or reactions regarded by the describer as unique but whose identity is so completely lost in large collections among series of closely related strains as to make them unidentifiable by description—are listed in the check list without characterization.

SPECIES KEYS

No general or comprehensive key to the species of *Aspergillus* is presented. Instead, a series of comparatively simple species keys are included in the discussions of the several groups. The recommended procedure in identifying an *Aspergillus* with the aid of this manual is first to determine its group relationship by means of one or more of the group keys presented below, then assign the culture more precisely to species by means of the intra group species key for the particular group to which the form belongs.

GROUP KEYS

Three keys to the groups of *Aspergilli* are offered. The first is presented in the form of a diagram and presents the different groups in what we consider their natural order. Presentation of this key in graphic form it is believed, will materially assist the user in grasping the various characters which ally and interrelate the different groups. Primary separation is based upon the number of series of sterigmata, whether single or double. Secondary separation is based upon the character of the conidiophore whether rough or smooth. Tertiary separations are based upon the presence or absence of perithecia, hulle cells, and sclerotia and upon the color of the conidiophore wall.

In assigning species to groups by means of this key, the transitional or intermediate character of certain species becomes strikingly apparent. For example *Aspergillus caespitosus* possesses the brown conidiophore and conidial coloration of *A. nidulans* furthermore it produces clusters of irregular, thick walled hulle cells but the head is radiate or only loosely columnar and no perithecia or ascospores are produced. It is placed in the *A. nidulans* group with full recognition that it possesses certain characters which relate it to *A. ustus*. *Aspergillus alliaceus* is another form with intermediate characters. The conidiophore is uncolored and smooth when examined in liquid mounts (appearing finely roughened when examined dry) and the sclerotia are black but the heads are essentially ochraceous in color. It is placed in the *A. uentii* group but shows unmistakable relationship to *A. ochraceus*. *Aspergillus sparsus* is a species of uncertain relationship. It possesses a conspicuously roughened yellow conidiophore and globose head, and is placed in the *A. ochraceus* group but the conidial heads show a greenish color which is not found in any other known member of this group while the character of the conidiophore will not warrant placement elsewhere. The so-called bronze series in the *A. tamaris* group is transitional in the direction of *A. flavus*. Colonies are conspicuously green when young and retain a greenish tint for a considerable period in contrast to *A. tamaris* which never shows true green and appears greenish only transiently when young. Such a list of intermediate species and forms

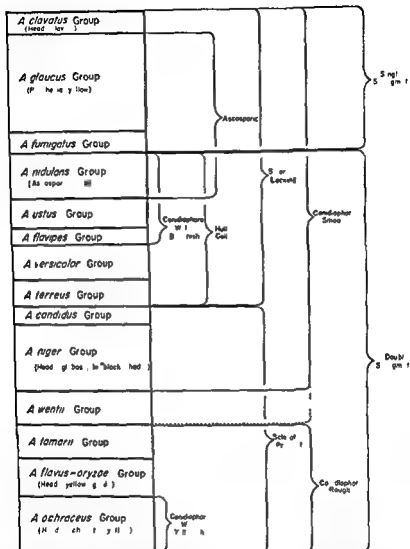


FIG. 20. Graphic representation of natural relationships among groups comprising the genus *Aspergillus*. The abundance of species in the different groups is roughly indicated by the size of the group boxes in the figure.

could be extended but sufficient have been cited to indicate that the various groups like the species which comprise them often cannot be set apart by sharp lines of demarcation.

In using this manual, and in studying the *Aspergilli* generally it is important that the worker should realize that these organisms vary within the species the species vary within the group, and to a lesser extent, the groups themselves vary within the genus. In other words, nature did not realize that we were going to write this manual when the various species and groups were being developed, hence not all of the forms one encounters will fit into the various compartments which have been constructed, although these are on the whole, comparatively elastic. This can be illustrated in another way. If we completely disregard color the genus *Aspergillus*, as depicted in figure 20 can be likened to the spectrum. There is a green region and a yellow region in the spectrum and the two regions are, on the whole distinct. Furthermore, within each of these certain fixed and definite lines can be identified. It is, however extremely difficult if not impossible to say where the green region ceases and the yellow begins. So it is with the species and groups of the *Aspergilli*. There is a definite *nidulans* group and a definite *ustus* group, but the line separating the two is extremely tenuous.

We do not in any sense infer that the *Aspergilli* cannot be classified—that they cannot be separated into groups, species, and even varieties. This manual is evidence that they can. But we do wish to emphasize that we are dealing with living and variable organisms, and that in describing them, we should be as explicit as possible and still keep our concepts reasonably elastic.

Key to Groups—Based Primarily Upon Color

The second key is based primarily upon color and is entirely artificial in its construction. The various groups are separated by contrasting coloration and closely related groups may appear widely separated in the key. In practice such a key is very useful since color is the most obvious character of an *Aspergillus* and since the species comprising a particular group with but few exceptions are characterized by variations in shade of color rather than differences in basic coloration. Presumptive assignment of the *Aspergilli* to groups can usually be made from this type of key which is based primarily on color supplemented by the use of a handlens or dissecting microscope.

- | | | |
|----|---|-----------------------|
| A | Conidial heads in definitely green, blue green, or yellow green shades in young fruiting colonies | B |
| AA | Conidial heads lacking green colors (Greenish in exceptional cases) | K |
| B | Conidial heads in green and blue green shades | C |
| BB | Conidial heads in yellow green shades | A <i>flavus</i> group |

C	Conidial stalks and heads coarse—heads clavate	<i>A. clavatus</i> group
CC	Heads not clavate	D
D	Colonies mostly showing yellow perithecia and more or less yellow and red hyphae	<i>A. glaucus</i> group
DD	Colonies lacking yellow perithecia and more or less yellow and red hyphae	I
E	Colonies producing columnar spore masses	F
EE	Colonies producing radiate globose or hemispherical heads	H
F	Rapidly growing and spreading colonies	G
FF	Slowly and restrictedly growing colonies	<i>A. restrictus</i> series
G	Conidial columns long narrow	<i>A. fumigatus</i> group
GG	Conidial columns short and broad perithecia usually present, ascospores red	<i>A. nidulans</i> group
H	Heads radiate in blue green dull green to pale tan or flesh-colored shades	<i>A. versicolor</i> group
HH	Heads in some other color	L
h	Heads in long compact columns avellaneous to cinnamon shading toward colorless through light flesh colors	<i>A. terreus</i> group
hA	Heads in some other color	L
L	Colonies more or less floccose heads in dull olive grays to fuscous	<i>A. ustus</i> group
LL	Heads in some other color	M
M	Young heads white or only slightly tinged in age	N
MM	Heads in some other color	O
N	Young heads white usually in short columns broadening at apex often becoming avellaneous in age	<i>A. flammipes</i> group
NN	Heads persistently white larger heads definitely globose or radiate	<i>A. candidus</i> group
O	Heads in sulphur yellow to ochre shades	<i>A. ochraceus</i> group
OO	Heads in some other color	P
P	Young colonies showing a greenish color passing into brown	<i>A. tamaris</i> group
PP	Heads not showing greenish	Q
Q	Heads in purple brown to black shades	<i>A. niger</i> group
QQ	Heads in yellowish brown shades orange to deep brown to umber color	<i>A. wentii</i> group

Key to Groups—Based Primarily Upon Morphology

The third key is based primarily upon morphology, with colony color employed as an accessory differentiating character. This key is also artificial in construction and is designed to separate the various groups by the simplest and most direct means possible. Groups naturally related may or may not appear in their proper sequence. With the data developed upon the descriptive sheet (p. 82), most of the *Aspergilli* can be traced to their proper placement in classification schemes by using the following key. Natural arrangement is disregarded and the same group is occasionally reached in different places in the key.

- | | | |
|----|--|------------------------------------|
| A | Species producing perithecia and ascospores | II |
| AA | Species not producing perithecia and ascospores | D |
| B | Ascospores colorless | C |
| BB | Ascospores purple red | A <i>nidulans</i> group |
| C | Perithecia white to flesh color enmeshed in a loose net work of colorless hyphae | A <i>fischeri</i> |
| CC | Perithecia yellow to orange naked vegetative hyphae often showing red to orange granules | A <i>glaucus</i> group |
| D | Conidial heads cylindrical clavate vesicles definitely clavate | A <i>claratus</i> group |
| DD | Conidial heads not cylindrical clavate | E |
| E | Colonies showing green or greenish color at some stages of development | F |
| EE | Colonies lacking green color | F |
| F | Conidiophore wall rough or pitted | G |
| FF | Conidiophore wall smooth | H |
| G | Colonies green or yellow green to yellowish | A <i>flavus</i> or <i>ae</i> group |
| GG | Colonies greenish brown when young becoming rich brown or umber in age | A <i>tamarii</i> group |
| H | Sterigmata in one series | I |
| HH | Sterigmata in two series | I |
| I | Conidia elliptical to pyriform | J |
| II | Conidia spinulose 2.5 to 4 μ globose chains in compact columns | I <i>fumigatus</i> group |
| J | Colonies mostly showing yellow perithecia sterigmata usually coarse | A <i>glaucus</i> group |
| JJ | Colonies lacking perithecia conidia in narrow columns | I <i>restrictus</i> group |

THE USE OF THE MANUAL

91

K	Conidiophores in yellow brown shades	L
KK	Conidiophores not colored	O
L	Conidial heads definitely green	M
LL	Conidial heads greenish only when young then in yellow brown shades	t <i>ustus</i>
M	Ascospores produced—purple red in color	A <i>nidulans</i> group
MM	Ascospores not produced	\
N	Colonies showing irregularly clustered hülle cells	t <i>caespitosus</i>
NN	Colonies not showing hülle cells bright green spreading	t <i>unguis</i>
□	Conidial area in dull green shades (sometimes partially or completely replaced by tan)	t <i>versicolor</i>
OO	Conidial area in blue greens	A <i>sydoux</i>
P	Conidiophore walls smooth	Q
PP	Conidiophore walls rough	v
Q	Conidiophore walls pale yellow in outer layer heads white when young often becoming avellaneous in age	t <i>flavipes</i>
QQ	Conidiophore walls colorless or partially yellow brown near the head	R
R	Conidial chains in solid columns compact at base	S
RR	Conidial chains radiate at least in the larger and typical heads	T
S	Conidial heads in avellaneous shades	A <i>terreus</i>
SS	Conidial heads flesh color (in pinkish shades)	t <i>carneus</i>
T	Heads white or tardily in yellowish shades	t <i>candidus</i> group
TT	Heads in darker colors	U
U	Conidial heads globose in purple brown to black (rarely brown or paler)	A <i>niger</i> group
UU	Conidial heads globose in yellowish yellow brown and dark brown shades	A <i>wentii</i> group
	Conidiophore walls rough yellow heads yellow to ochre	A <i>ochraceus</i> group

CHAPTER VIII

THE ASPERGILLUS CLAVATUS GROUP

Outstanding Characters

- Conidial heads clavate¹, large pale blue green
- Conidiophores generally coarse, smooth walled, uncolored
- Sterigmata in one series
- Conidia elliptical smooth comparatively thick walled

Group Key

- Conidial structures not exceeding 40 mm in length
Aspergillus clavatus Desm
- Conidial structures often 1 to 5 or more cm in length
Aspergillus giganteus Wehmer

Aspergillus clavatus Desmazieres in Ann Sci Nat Bot (2) 2 71, p 2, fig 4 1834

Colonies upon Czapek's solution agar growing rapidly at 20-24° C plane or slightly furrowed in certain strains tending to become floccose but generally characterized by a surface mycelial mat and abundant erect conidiophores up to 30 mm in length bearing large blue green clavate conidial heads evenly distributed or arranged in more or less well defined zones (Pl III A and Fig 21 A), reverse generally uncolored but becoming browned in age in some strains odor strongly foetid in some strains not pronounced in others Conidial heads clavate large commonly ranging from 300 to 400 μ by 150 to 200 μ in age splitting into 2, 3 or more divergent columns of compacted conidial chains (Fig 21 B) approximately slate olive in color (Ridgway Pl XLVII) Conidiophores 15-30 mm in length 20 to 30 μ in diameter comparatively thin walled smooth colorless gradually enlarging at the apex into a clavate vesicle which is fertile over an area up to 200 to 250 μ in length and 40 to 60 μ or more wide (Fig 21 C) Sterigmata in a single series varying in size from 2.5 to 3.5 μ by 2.0 to 3.0 μ at the base of the vesicle to 7.0 or 8.0 and occasionally 10 μ by 2.5 to 3.0 μ at its apex (Fig 21 D) Conidia elliptical comparatively

¹ *Aspergillus jonus* Raper and Thom (see page 187) is characterized by a smaller clavate vesicle in one of its conidial phases It is hardly to be confused with the clavatus group however because of the whiteness of its conidial masses the double series of sterigmata and the intermixture of more or less abundant green A sydow-like heads in cultures at room temperature

heavy walled smooth 30 to 40μ by 20 to 30μ occasionally larger in some strains and irregular in others

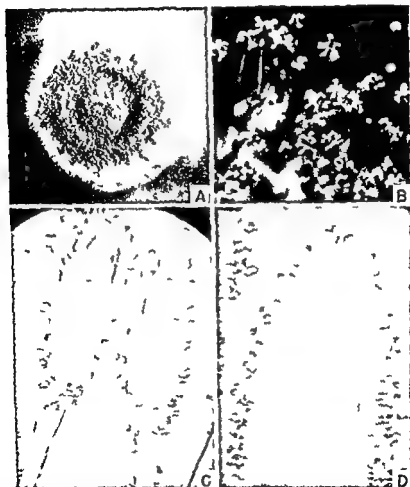


FIG. 21 *Aspergillus clavatus* Deam. A Colony growing on Czapek's solution agar 10 days room temperature $\times 13$ (Thom No 5169). B Conidial heads showing characteristic splitting in age $\times 12$ (Strain NRRL No 1873). C Conidial heads showing characteristic clavate form of vesicle $\times 180$ (Strain NRRL No 6). D, Portion of a conidial head further enlarged showing closely packed single series of sterigmata $\times 600$ (Strain NRRL No 6).

Cosmopolitan in distribution and especially common in soil decaying vegetation dung and other materials where active decomposition of nitrogenous materials is taking place. Cultures examined include numerous

strains from various parts of the United States together with isolations from China British Guiana, Cuba Panama and European sources

The species description as presented is based upon a large number of closely related strains that have been examined and is adequately represented by such specific strains as NRRL Nos ■ 5 8 and others

Upon malt extract agar details of morphology and colony characteristics may or may not conform with those listed above For example, in such typical strains as Nos 2 5, and 8 conidial structures are generally more abundant upon malt than Czapek's agar and may average as much as 20 to 25 percent larger in size In other strains such as Nos 4 and 6 ■ markedly different response is noted upon this medium Conidial heads, although



FIG 22 *Aspergillus clavatus* Desm (Strain NRRL No 1 Thom No 107) A Colony growing upon Czapek's solution agar 10 days room temperature X 13 B Conidial heads diminutive and somewhat atypical but showing characteristic clavate form X 600

greatly increased in number are much reduced in size with conidiophores generally 1 mm or less in length bearing heads only 100 to 125 μ long and proportionately reduced in diameter In these latter forms conidia are somewhat irregular in form and generally larger than in the more typical strains first considered

Culture NRRL No 1 (Thom No 107) differs markedly from the species description in producing deeply floccose colonies (Fig 22 A) and comparatively few spore heads which are extremely variable in size These range from very small fruiting structures (Fig 22 B) borne as branches upon aerial hyphae to structures arising from the substratum which are characterized by dimensions almost typical of the species This culture is more nearly normal upon malt than upon Czapek's solution agar but ■ unique

among the strains examined by us and must be considered somewhat atypical. It deserves particular attention because it was obtained from the Centraalbureau in Baarn in 1908 as *Aspergillus clavatus* Desm. and has undoubtedly been widely distributed by that organization—in fact it is quite probable that it has been examined by more investigators than any other strain belonging to this group. We refrain from using it as a basis for the species description however despite its classic history since the less floccose and more heavily sporing strains are so much more commonly encountered in nature.

Members of this species produce a strong alkaline reaction upon many culture media and this is usually associated with a strong foetid odor. For example when grown upon Czapek's solution agar containing only NaNO_3 as nitrogen and sucrose as a carbon source the reaction of typical strains may reach pH 9.5 or even higher accompanied by a strong odor of trimethylamine almost approaching putridity. No other species of *Aspergillus* is known to react in this manner although some strains of *A. flavipes* give some suggestion of it. The ability to produce and more particularly to withstand strong alkaline conditions undoubtedly accounts for the common occurrence of this species upon dung and other nitrogen rich substrata undergoing decomposition.

Aspergillus giganteus Wehmer in Central f. Bakt. etc. 2 Abt. 18 No. 13/15 385 1907

Colonies upon Czapek's solution agar growing rapidly at 20°C characterized by an extensive surface and submerged vegetative mycelium and an early development of abundant conidiophores 20 to 40 mm high followed by the subsequent development of less numerous conidiophores ranging up to several centimeters in length (Pl. III B and Fig. 23 A) the latter strongly phototropic and generally more abundant in marginal areas commonly obscuring the more central mass of short conidiophores. Colonies at first white becoming pale blue green as conidial heads mature reverse dull tan becoming brown in age odor none to somewhat foetid in certain strains. Conidial structures varying greatly in dimensions and falling for the most part into two general size ranges: (1) conidiophores commonly 2 to 3 mm rarely exceeding 4 mm in height bearing clavate heads 200 to 350 μ in length; (2) conidiophores one to several centimeters in length bearing heads up to 1 mm in length. The relative proportions of these head types is strongly influenced by environmental conditions and specific strain characteristics. Conidial heads pale blue green in age splitting into 2 or more columns extending the length of the vesicle (Fig. 23 B). Vesicles consisting of the expanded terminus of the conidiophore ranging from 100 to 250 μ by 30 to 50 μ upon short conidiophores to 400 to 600 μ by 120 to

180 μ upon long conidiophores (Fig 23 C) Sterigmata in a single series ranging from 30 to 40 μ by 25 to 30 μ at the base of the vesicle to 60 to

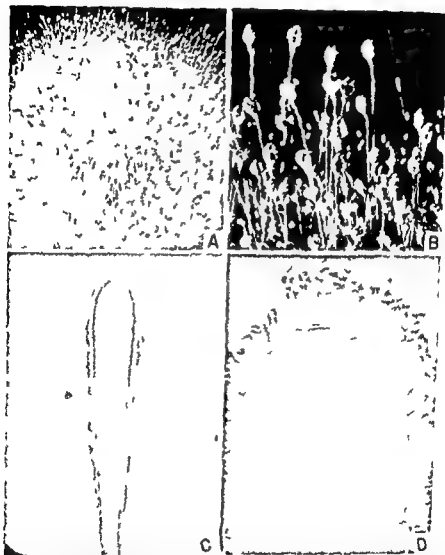
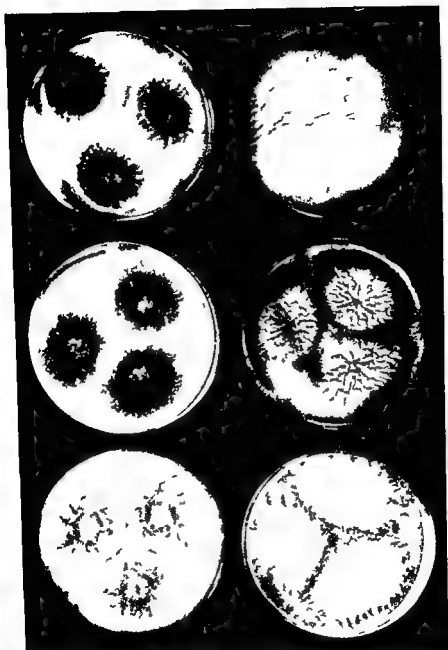


FIG 23 *Aspergillus giganteus* Wehmer (Strain NRRL No 10) A Portion of colony on Czapek solution agar showing characteristic long conidiophores and large clavate heads 10 days room temperature $\times 13$ B Portion of colony margin somewhat enlarged $\times 11$ C Single conidial head showing the very elongate vesicle characteristic of the species $\times 130$ D Terminal portion of vesicle showing the closely crowded single series of sterigmata $\times 600$

8.5 μ by 2.8 to 3.5 μ at the apex (Fig 23 D) . Conidia elliptical thick walled smooth 3.5 to 4.5 μ by 2.4 to 3.0 μ



Pl. III

4: ppe left: 1 ppe right: 1 out. Deam NRRLN 8 B (upper right) 4 ppe gillia ggontra. W hme
 NRRLN 111 1st 1st 20 C ne-a ded 11 m nat C free to 1 ft) A ppe R pens (Cl)
 d B 3 NRRLN 71 D (to to right) 4 ppe R vuler item NRRLN 54 E (low left) 4 ppe i
 1 11 d m (M) Th m d Cl ret NRRLN 90 F (to to right) A ppe gillia or glaucus Thom
 d Rape NRRLN 12 E g row A 1 B grow g po ta d d C pek act with 3 ppe t
 ne C to f ro g po C f k act to g with 20 ppe t rose (Col ph: rapha by 11 es
 N rt) R gonal Rees 8 Laboratory Reprod ced thro gh co-operatio f Chas Fize & Co I)

The above species description is centered upon strain NRRL No. 10 (Thom No. 5581 13A) isolated from Yucatan caves by Prof. F. A. Wolf (1938). Additional strains examined include isolations from Texas, Illinois, Mexico, Puerto Rico, and strain NRRI No. 1725 (Thom No. 138) received from Dr. Westerdijk in 1910 as *A. giganteus* Wehmer.

In the present treatment we include under the species name *A. giganteus* Wehmer all strains which produce conidiophores in excess of 1 cm. in length. While this may appear somewhat arbitrary, it is done since members of the *A. clavatus* group seem to fall into two natural series: (1) those which never produce conidiophores in excess of 5 to 6 mm. in length irrespective of environmental conditions or medium composition, and (2) those which regularly produce few to many very long-stalked fruiting structures under the usual conditions of laboratory cultivation and examination. To the first of these series is applied the species designation *A. clavatus* to the second *A. giganteus*.

Strains of *A. giganteus* like those of *A. clavatus* differ materially in their growth and cultural appearances upon different culture media. More striking, however, is their response to light and temperature. This has been observed by Wehmer (1907), Wolf (1938), and others, and has been studied somewhat exhaustively by Webb (1942). Using the Wolf isolate, Webb found that the production of long conidiophores was favored by cultivation upon media containing from 1 to 10 percent sucrose and incubation at 20° C. in the presence of light or darkness, whereas heavy conidial production and the development of short conidiophores were favored by incubation at 30° C. in darkness. Different strains vary materially in their cultural appearance upon such standard media as Czapek's solution agar and can be roughly grouped into three sub-series as follows: (1) wholly typical strains consistently producing the cultural picture as defined for the species; (2) strains producing an unusually heavy crop of short-stalked conidial structures in colony centers, followed by the production in marginal areas only of long-stalked fruits typical of *A. giganteus*; and (3) rather sparsely growing strains in which there is a general admixture of short-stalked and scattered long-stalked fruits ranging up to 2 to 3 cm. in length. The latter group is considered as possibly representing atypical and somewhat depauperate strains of the first. The second seems to constitute a consistent and fairly well-defined cultural entity, but does not differ from typical forms sufficiently to warrant separation as a variety.

The validity of the species *A. giganteus* has been questioned by some authors. Blochwitz (1929) regarded III as a mutation of *A. clavatus* and so designated it in his monograph of the *Aspergilli*. His view may be correct. It is our belief, however, that the species should be retained since forms producing the giant conidiophores noted by Wehmer are repeatedly

isolated from nature, and since no evidence has been presented indicating that these larger forms arise directly from the smaller and more common forms. While it is true that conditions can be altered so that *A. giganteus* cultures suggest *A. clavatus*, no one has yet demonstrated that the reverse can be accomplished.

Group Synonyms

The following names have been proposed for specimens belonging to this group but without adequate data to warrant recognition as valid species.

A. clavellus Peck in N. Y. State Mus. Nat. Hist. Rept. III 49 Pl. III figs 1-5 1881. Described from cooked squash in New York State. No data is presented which would warrant separation of this form from *A. clavatus* Desm.

A. nestendorpii Sacc. and March. in Rev. Mycologique 7 149 1885. Was listed from cow dung. Correctly assigned to *A. clavatus* by Lindau in Deutsch. Krypt. Fl. Pilze 8 152 1907.

A. fusco-cinereus Ellis and Morgan was the name attached to Morgan's jacket No. 674 showing a very small clavate aspergillus which has not been collected again hence never cultivated. It was probably some member of this group.

A. pseudo-clavatus Purjewitch in Schrift. Naturforsch. Gesell. Kiev 16 (2) 300 pl. III 1900. See also Sacc. Syll. 16 1028. The organism in culture was reported as having both primary and secondary sterigmata in a small sized clavatus type of head and perithecia with ascospores which were not adequately described. Until somebody finds this organism again and reports its cultivation and more complete description it will remain doubtful. (See Thom and Church. The Aspergilli p. 100 1926.)

Occurrence and Economic Importance

Members of the *A. clavatus* group are quite common in soils and decomposing materials characterized by a comparatively high nitrogen content. They appear to be common upon the dung of various animals and in the writers' experience have been isolated repeatedly from that of chickens. While the subject has not been adequately investigated it is probable that the ability of members of this group to withstand strongly alkaline conditions enables them to operate successfully as agents of decomposition in situations where almost all other fungi are eliminated.

Antibiosis

Certain strains of *A. clavatus* produce substances in the substratum which are capable of destroying *Staphylococcus* and other microorganisms. Such activity was first reported by Weisner in March 1942 (Nature 149 p. 356) and subsequently by Waksman, Horning, and Spencer in August of the same year (Science 96 p. 202). To the active substance the latter investigators assigned the name *clavacin* and noted that it appeared similar to if not identical with that studied by Weisner (1942) to which the designation

clavatin has since been applied. Additional studies by Waksman and his co workers have further defined its action and enlarged the list of bacterial species inhibited (1942b and 1943). Hooper et al (1944) have demonstrated the identity of *clavacin* and *patulin* a bactericidal substance obtained from *Penicillium patulum* by Raistrick and associates (1943) and reported to be of value in the treatment of the common cold.

Philpot (1943) reported the production of a penicillin like substance by a strain of *Aspergillus giganteus*. In earlier tests by Wilkins and Harris (1942) this strain had been found to produce a substance active against *Staphylococci*.

CHAPTER IX

THE ASPERGILLUS GLAUCUS GROUP¹

Outstanding Characters

- Perithecia generally present, yellow, globose to subglobose, thin walled, suspended in networks of red or yellow hyphae
- Asci 8 spored, without definite arrangement usually ripening in 2 to 4 weeks
- Ascospores lenticular, smooth or rough walled, generally showing an equatorial line or furrow with or without flanking ridges or crests
- Conidial heads more or less abundant, radiate to somewhat columnar, typically in some shade of green
- Conidiophores smooth walled terminating in dome like vesicles
- Sterigmata in one series rather coarse
- Conidia elliptical to subglobose; uniformly and characteristically roughened

General Considerations

Aerial hyphae encrusted with yellow orange or red granules are abundant in perithecial areas of most of the strains of the group. Both laboratory cultures and naturally moldy specimens frequently show this as their most conspicuous character one which is readily recognized with the hand lens. In nature, molds of this group appear as patches of green yellow reddish or reddish yellow mold depending upon the relative abundance of conidial heads perithecia and encrusted aerial hyphae and especially influenced by the composition of the substratum.

Representatives of the *A. glaucus* group are universally distributed in nature and are significant in the incipient spoilage of many organic materials useful to man. They occur particularly upon products characterized by a high osmotic tension such as preserves jams cured meats leather goods improperly dried hay moist grain and soft woods stored under humid conditions. The classic habitat is improperly dried herbarium specimens.

The earliest references to any *Aspergilli* concern representatives of this group, for botanists early encountered them upon herbarium material. Micheli in 1729 used the generic name *Aspergillus* (rough head) for the conidial heads characterized by divergent chains of spores commonly present upon such specimens. Later in the century Wiggers (1780) pro

¹ Abridged from Charles Thom and Kenneth H. Raper *The Aspergillus Glaucus Group* U S Dept of Agr. Misc. Pub. No. 426 Washington D. C. September 1941

posed the name *Mucor herbariorum* for the yellow penithecia found mixed with the *Aspergillus* heads which he regarded as a different mold. In 1809 Link designated the green heads *Aspergillus glaucus* and the yellow penithecia *Eurotium herbariorum*. Half a century later DeBary (1854) proved that the *Aspergillus* heads and *Eurotium* penithecia were borne upon the same mycelium hence were one fungus. Although it could be maintained that the name *Eurotium* (designating the perfect stage) should take precedence over *Aspergillus* (descriptive of the conidial apparatus) most recent authors have tended to go back to Micheli and use the name *Aspergillus* for the whole group because of the obvious relationship of many conidial forms for which no penithecia are known.

Laboratory Cultivation

The pattern and size of the ascospore when present is especially significant in describing species of the *Aspergillus glaucus* group. Nevertheless the conidial apparatus and the vegetative mycelium of particular subgroups are so important that pure culture under known conditions is always desirable. The character of the colony as well as the amount of growth is strongly influenced by the culture medium and it is only upon substrata characterized by a high osmotic tension that typical penithecia and conidial heads are produced. It should be noted however that characteristic heads and penithecia normally develop although few in number in situations where less concentrated media dry out rapidly as at the edge of an agar slant. Colony comparisons for correct identification can best be made in Petri-dish cultures in which direct observation with the compound microscope is feasible. Incubation at 22 to 25 C will permit the development of satisfactory colonies for descriptive study although the optimum for certain species is above or below this range as will be noted in connection with these descriptions and in the general discussion on the influence of temperature on colony growth and development in the genus (p. 45). Colony descriptions are based upon 3 week old cultures except as otherwise stated. For comparative culture the authors have followed Dale (1909) in using substrata containing high concentrations of sugar. The following formula is recommended.

Czapek's solution agar with 20 percent of sucrose

Sodium nitrate	3 gm
Dibasic potassium phosphate	1 gm
Magnesium sulfate	0.5 gm
Potassium chloride	0.5 gm
Ferrous sulfate	0.01 gm
Sucrose	200 gm
Agar	15.0 gm
Dist. water	1 000 cc

Group Limits and Relationships

Exceptional strains lacking perithecia but presenting conidial morphology clearly belonging to the *A. glaucus* group in its strictest sense are occasionally found. In addition the *A. restrictus* series (*A. penicilloides* series of George Smith, 1931) shows conidial morphology clearly related to the group, but differing markedly from the usual types in colony coloration and in the absence of perithecia. Thom and Church (1926) considered these latter types as representing intermediate forms between the *Aspergillus glaucus* and *A. fumigatus* groups. In the present treatment, we include them as non ascospore and for the most part diminutive forms sufficiently related to the ascospore species to be included with them in the *A. glaucus* group.

Certain other groups of the *Aspergilli* present characters suggesting those of the "glaucus" group considered here. The ascospores of *A. fischeri* Wehmer (See *A. fumigatus* group) and of the *A. nidulans* group (which see also Thom and Raper, 1939) in general resemble those of the *A. glaucus* group but the yellow perithecia suspended by yellow and red encrusted hyphae do not occur outside of this group.

Group Key (Based Primarily Upon Perithecia and Ascospores)

I Perithecia present

A Ascospores lenticular 6 μ or less in long axis

1 Ascospores with convex faces smooth (or nearly so)

a Equatorial ridges lacking furrow absent or showing only as a trace
A. repens series

b Equatorial ridges low and rounded furrow broad and shallow
A. ruber series

c Equatorial ridges thin and flexuous crestlike (spore resembling a pulley)
A. cheilasteri series

2 Ascospores with convex faces rough *A. amstelodami* series

II Ascospores lenticular 6 μ or more in long axis

Large spored species or the (*E.*) *herbariorum* series

II Perithecia absent

A Colonies predominantly in yellow orange to brownish shades

1 Heads approximating those of *A. repens* *A. argillaceus* Bourge^{*}

II Heads proliferating giving rise to many subheads
A. proliferans G. Smith

B Colonies in dark green or blue green shades

1 Restricted velvety with short conidiophores and abundant columnar heads
A. restrictus series

2 Spreading floccose with long conidiophores and globose heads
A. staconicus Kinoshita

* Culture distributed by Bourge as a new species. Believed to represent only a non ascospore strain of *A. repens* hence not recognized as a valid species by the writers (see p. 111).

THE ASPERGILLUS GLAUCUS GROUP

THE ASPERGILLUS REPENS SERIES

Ascospores lenticular, mostly 4.8 to 5.4 μ by 3.8 to 4.4 μ smooth walled with equatorial area rounded or somewhat flattened and occasionally indented showing a trace of furrow but without crests or ridges

The *Aspergillus repens* series as based upon the ascospore described includes a great number of universally distributed strains which retain some cultural individuality. Consequently several of them have been described as species by earlier workers. From comparison of a great series of these forms it seems necessary to bring together under the name *A. repens* (Cda) DeBary, a very considerable number of forms (some of them regarded as species by others) in which the ascospore is typical for the group and the colony difference falls within lines of quantitative rather than qualitative variation.

The following key is offered as a means of separating culturally distinct strains or groups of strains

A Conidial heads large borne above the surface layer of perithecia and enveloping hyphae

- 1 Heads radiate long-stalked
- 2 Heads columnar short-stalked

A. repens (Cda) DeBary
A. dierezi Bourge

B Conidial heads small enmeshed with the perithecia in a felt of sterile hyphae

- 1 Felt orange yellow loose textured radially wrinkled
- 2 Felt yellow buff close textured plane or nearly so

A. pseudoglaucus Bloch
A. profusus Hann

Aspergillus repens (Cda) DeBary in Abhandl. I Senkenberg Naturf. Gesellsch. 7: 379. 1870

Synonyms *A. glaucus* var *repens* Cda. Icones Fungorum 5: 53. Taf. II fig. 27. 1842

A. scheelei Bain and Sart. Soc. Mycol. de France. Bul. Trimest. 28: 257-262. pl. V. 1912

A. B var *scheelei* Bain and Sart. Soc. Mycol. de France. Bul. Trimest. 28: 262-267. pl. VI. 1912

Colonies upon Czapek's solution agar (3 percent sucrose) restricted plane or somewhat wrinkled forming a rather compact felt (fig. 24 A₁) with the marginal area near Scheele's green (Ridgway Pl. VI) from enveloping heads older areas yellow green to greenish gray and enmeshing large numbers of aborted perithecia producing few ascospores normal perithecia found only when such colonies spread over the bare walls of the vessel. Reverse in shades of greenish yellow at colony margin to deep maroon or almost black in older areas.

Species name not recognized as valid by authors of this publication

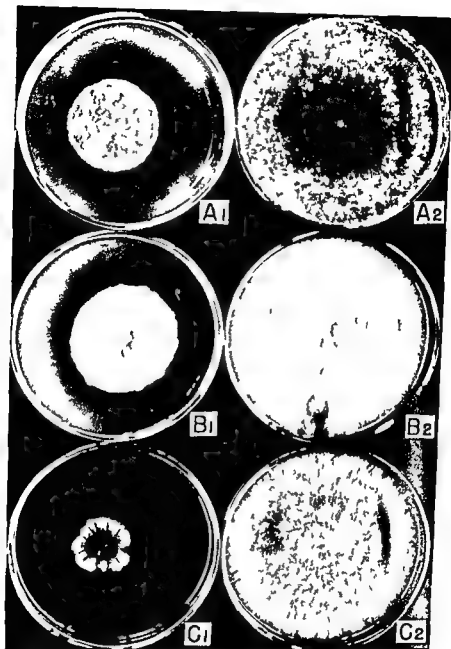


FIG. 24. Comparative growth of *Aspergillus repens*, *A. chevalieri* and *A. ruber* upon two different culture media: three weeks incubation at room temperature. *A. 1* and *2* *A. repens* NRRL No. 17 upon (1) Czapek's solution agar (3 per cent sucrose) and (2) Czapek's solution agar with 20 per cent sucrose. *B. 1* and *2* *A. chevalieri* NRRL No. 18 and *C. 1* and *2* *A. ruber* NRRL No. 52 upon the same media in similar arrangement.

Colonies upon Czapek's solution agar with 20 percent of sucrose spreading broadly and rapidly, plane or slightly wrinkled, orange yellow, commonly characterized by broad zones of dull green conidial heads (Pl. III C and fig. 25 A and B), surface growth consisting of loosely woven hyphae studded with orange granules, enmeshing abundant yellow perithecia above.

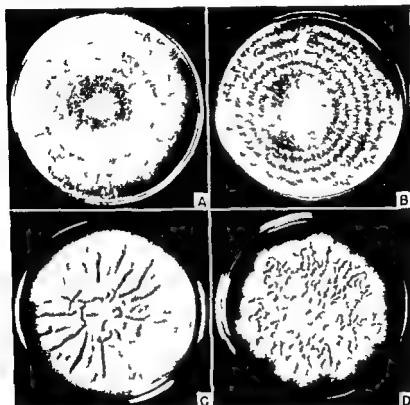


FIG. 25. Comparative growth of members of the *Aspergillus repens* series upon Czapek's solution agar containing 20 percent of sucrose, incubation at room temperature for three weeks. A and B, Typical cultures of *A. repens*. C, *A. pseudoglaucus* NRRL No. 40. D, *A. pseudoglaucus* NRRL No. 45.

which project abundant conidial heads, the whole colony and especially the marginal areas and adjacent wall of the culture dish commonly overgrown by a loose arial network of hyphae bearing conidial heads and scattered perithecia, reverse varying from yellow-orange to deep maroon.

Perithecia very abundant, borne in loose networks of yellow to orange red hyphae (fig. 26 A), yellow, spherical to subspherical, mostly 75 to 100 μ .

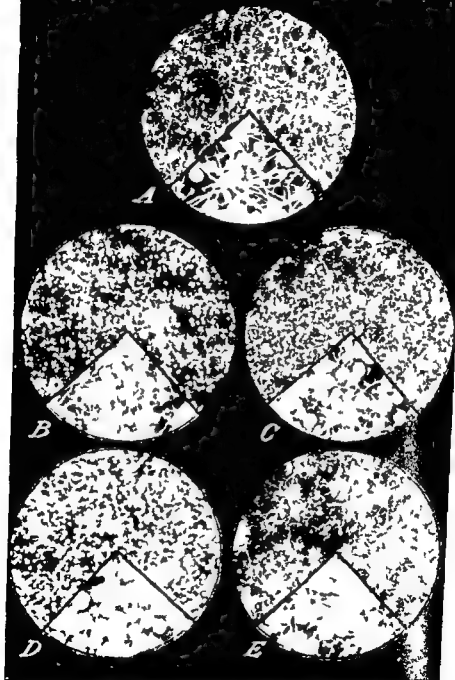


FIG. 26. Marginal areas of colonies of representative species of the *Aspergillus glaucus* group showing the relative size and arrangement of perithecia (conidial heads not in focus). A *Aspergillus repens* B *A. chervilieri* C *A. ruber* D *A. amstelodami* E *A. echinulatus*. Figures $\times 10$; inserts $\times 30$. (Reprinted from Thom and Raper. *The Aspergillus glaucus* Group. U.S.D.A. Misc. Pub. 426. 1-45. 1941.)

THE ASPERGILLUS GLAUCUS GROUP

occasionally up to 125μ asci 10 to 12μ ascospores lenticular mostly 4.8 to 5.6μ by 3.8 to 4.4μ smooth walled with equatorial area rounded or somewhat flattened and occasionally indented showing a trace of furrow but without crests or ridges (fig 27 A) Conidial heads abundant varying in different strains from 125 to 175μ in diameter consisting of diverging chains of conidia radiating from a hemispherical vesicular apex of the conidiophore (fig 28 A), conidiophores smooth mostly colorless 500 to 1000μ in length broadening at the apex to a vesicular area about 25 to 40μ in diameter sterigmata in one series 7 to 10μ by 3.5 to 4.5μ conidia elliptical to subglobose spinulose mostly 5 to 6.5μ

Represented by cultures NRRL No 12 No 17 and more than a score of others included in this study In this connection it should be noted that of 37 cultures examined in the present study that produced ascospores characteristic of the *A. repens* series 29 produced colonies and microscopic details that place them in the species *A. repens* as described

This description is manifestly broad enough to include strains approximating the description given by Bainier and Sartory for *Aspergillus scheelei* and *Aspergillus B var scheelei* (1912b) Evidently *A. scheelei* was thought by the describers to represent a species with somewhat larger ascospores showing a more definite furrow whereas *Aspergillus B var scheelei* was a strain with smaller ascospores almost without a trace of furrow Both species were described as characterized by the production of a yellow pigment In the authors experience a distinction based upon color is largely invalidated by variants bridging the whole range from yellow-orange to deep orange red and even shades of brown when large numbers of strains of this series are compared in culture Strains also vary slightly in the pattern of their ascospores some rarely producing spores with a trace of furrow and others bearing a large proportion with such traces But among spores of a single strain limited variation in this character is normally encountered Thus the presence or absence of a slight furrow unless accompanied by significant differences in morphology or colony character would not seem to justify specific descriptions in this series

A strain designated as *Aspergillus dierckxi* presumably by Biourge but thus far unpublished was included in Gould and Raistrick's study of pigment production in the *A. glaucus* group (1934) As received from Raistrick's laboratory this organism (NRRL No 39) produces colonies showing no zonate arrangement of conidial heads Further heads are borne on shorter conidiophores than in typical *A. repens* and consist of columns of conidia rather than radiating chains Little or no red color appears in the colonies or in reverse Although no other strains showing exactly these differences have appeared in the authors collection separation as a distinct species is believed unwarranted

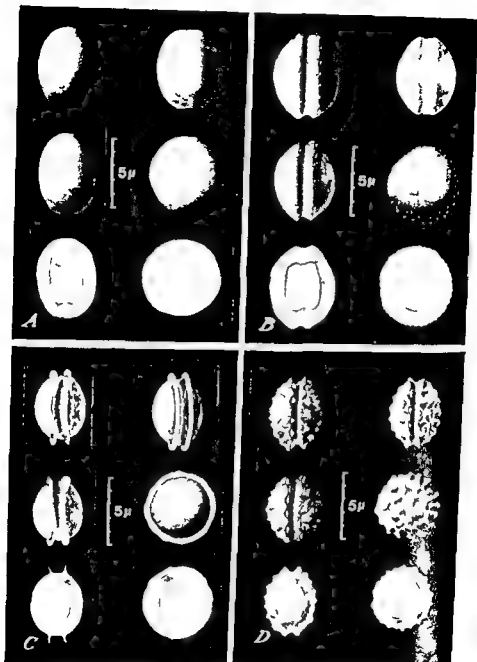


FIG 27 Ascospores representative of the four small spored series of the *Aspergillus glaucus* group A *A. repens* B *A. ruber* C *A. chevalieri* D *A. amstelodami*. In each species upper left and right and center left spores represent surface, profile views, center right, surface in face view, lower left, optical section in profile, and lower right, optical section in face view. (Reprinted from Thom and Raper, *The Aspergillus glaucus* Group, U. S. D. A. Misc. Pub. 426, 1-46, 1941.)

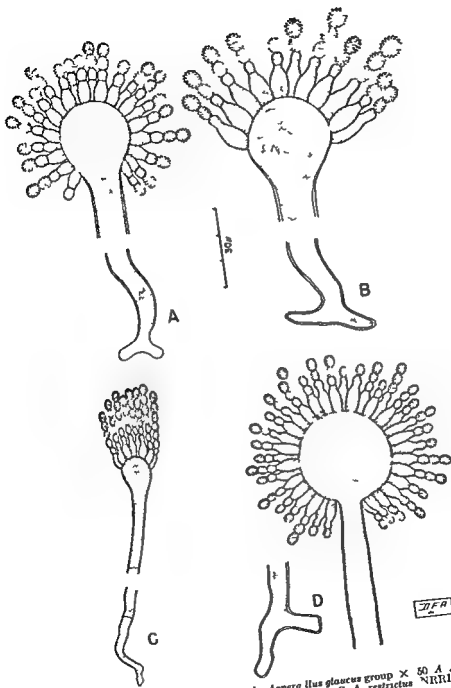


FIG 28 Conidial structures in the *Ispergillus glaucus* group $\times 50$ A *A. repens*
 NRRL No 21 B *A. echinulatus* NRRL No 131 C *A. restrictus* NRRL No 154
 D *A. taeniosus* NRRL No 161

Aspergillus pseudoglaucus Blochwitz, in Ann Mycol 57 207 1929
emend Thom and Raper, USDA Misc Publ No 426, p 12 1941

Colonies upon Czapek's solution agar (3 percent sucrose) restricted in growth radiately wrinkled yellow green to shades of gray, consisting of a mixture of small conidial heads, young or aborted perithecia and more or less colorless hyphae reverse orange at center becoming lighter toward the margin

Colonies upon Czapek's solution agar with 20 percent of sucrose spreading strongly wrinkled in a predominantly radiate manner, consisting of a felt of orange-encrusted hyphae encmeshing abundant perithecia orange except at margin where yellow green predominates from the presence of small conidial heads admixed with perithecia in the mycelial felt (fig 25 C), reverse yellow becoming orange brown or maroon in marginal areas

Perithecia abundant, spherical to subspherical, mostly 60 to 80 μ though occasionally 100 μ in diameter yellow, embedded in a felt of orange mycelium, asci 10 to 12 μ in diameter, ascospores lenticular 4.0 to 5.2 μ by 3.6 to 4.0 μ occasionally 5.6 μ in long axis smooth walled with equatorial region rounded or flattened without ridges, and with furrow generally lacking though occasionally showing as a trace Conidial heads few in number and generally submerged in the mycelial felt small mostly 50 to 75 μ in diameter but occasionally up to 100 μ , conidiophores mostly 150 to 300 μ in length 5 to 8 μ at the base broadening to a terminal vesicle 14 to 20 μ in diameter sterigmata in a single series 6 to 8 μ by 3 to 4 μ conidia subglobose, delicately spinulose, variable in size ranging from 5.5 to 7.5 μ in diameter

Represented in the NRRI collection by No 40 received from Baarn as *A. pseudoglaucus* Blochwitz and No 41 received from George Smith as *A. fumigatoides* Bain and Sart

There is reason to believe that the former culture is directly derived from Blochwitz's type It becomes necessary therefore to emend the description given by him insofar as the measurements and markings of ascospores and conidia are concerned Gould and Raistrick (1934) reported biochemical data upon a culture No A 38 received from Biourge as *A. fumigatoides* Bain and Sart (NRRL No 41) which is identical with *A. pseudoglaucus* (NRRL No 40) as sent to the authors by Westerdijk Obviously the culture from Biourge is incorrectly named It does not fit the species description nor the figures of *A. fumigatoides* (1909) in the size of its conidia the character of its perithecial wall or the pattern of its ascospores The ascospores of *A. fumigatoides* are shown as roughened over their entire surfaces as in *A. fischeri* whereas those of No 41 certainly belong in the *A. repens* series Distinctive colony characters however maintained stably through many transfers together with the size of the conidial apparatus warrant separating *A. pseudoglaucus* from *A. repens* and maintaining it as a species

A strain NRRL No 45 received from Dr B O Dodge and Miss Marjorie E Swift of the New York Botanical Garden in 1931 is characterized by an intensely wrinkled colony and a further reduction in the size and number of conidial heads (fig 25 D). Colonies are dull orange red and bear abundant perithecia enmeshed in a close felt of sterile encrusted hyphae. Obviously it should be considered with *A pseudoglaucus*.

In cultures received from Baarn (NRRL No 44) and from George Smith (NRRL No 45) as *A profusus* Hann (nomen nudum) there is a pronounced accentuation of the floccose habit already noted in *A pseudoglaucus*. Upon 20 percent sucrose Czapek agar these cultures which are obviously duplicates produce spreading plane or radiately wrinkled floccose colonies consisting of a close felt of light tan to buff-colored hyphae bearing occasional perithecia and widely scattered conidial heads. The perithecia are commonly embedded deep within the felt whereas the conidial heads are most evident at the colony margin. Although the ascospores of these strains are definitely of the *A repens* type they are generally flattened along their equators and commonly show a trace of furrow. An occasional ascospore shows a minute roughness in the equatorial region. The differences observed do not seem to warrant perpetuating the name *A profusus* and in agreement with Dr Westerdijk and coworkers (Centraal bureau List 1939) the cultures have been assigned to *A pseudoglaucus*.

Culture NRRL No 46 received from Raistrick in 1923 as *Aspergillus novus* Wehmer (nomen nudum) bears ascospores duplicating those of the cultures just considered. This strain is of particular interest, because in routine transfers colonies of two distinct types commonly appear. One of these is predominantly floccose and suggests the colonies of the strains received as *A profusus*. The other consists of a crowded surface layer of perithecia which is thinly veiled by a loose felt of orange red hyphae and in its gross appearance with the exception of its lighter color, is strongly suggestive of certain cultures of *Aspergillus ruber*. The authors agree with Wehmer (1901) and Blochwitz (1929a) that the species designation *Aspergillus novus* should be withdrawn.

A nonascosporic culture distributed by Bourge as *Aspergillus argillaceus* n. sp. was received upon two occasions from Prof. Raistrick's laboratory. From its appearance in culture and the morphology of its conidial structures this fungus would seem to represent a member of the *Aspergillus repens* series in which perithecial development has been wholly suppressed. Although it is questionable whether this fungus represents a true species a brief description is given because of its inclusion in biochemical studies by Raistrick and coworkers (1939, 1934, 1937). Colonies upon Czapek's solution agar with 20 percent of sucrose spreading irregularly consisting of a loose floccose felt of aerial hyphae and abundant conidial heads pale yellow green to clay color reverse yellow. Upon Czapek's solution agar

(3 percent sucrose) colonies restricted, raised in center, thinning toward margin, consisting of abundant conidial heads and interlacing hyphae, buff to clay colored, reverse yellow to tawny. Conidial heads abundant, dull green, up to 200μ in diameter and commonly splitting into fairly well-defined columns, conidiophores up to $1,000\mu$ in length. Conidia subglobose mostly 5.5 to 6.0μ but occasionally up to 7.0μ in long axis, spinulose.

ASPERGILLUS RUBER SERIES

Ascospores lenticular, 5.0 to 6.0μ by 4.0 by 4.8μ , colorless with broad, shallow furrow generally evident and flanked by low ridges, and with walls smooth except for minute roughness along the equatorial ridges.

This series includes a great number of strains showing variations in cultural appearance but producing ascospores of a limited size range and fairly well-defined pattern. For this particular study some 30 strains received from various culture collections and contributors and selected from the isolations made in this laboratory over a period of many years have been chosen for repeated culture and examination. Among these, many strains appear distinct but their differences are commonly bridged by intermediate forms. Separation within the series, therefore, must be along one of the following lines—either (1) strains must be separated upon minor characters, such as differences in the intensity of pigmentation, slight variations in ascospore character, etc., or (2) strains must be set off in broad and elastic subgroups, in some cases including large numbers which vary appreciably in detail. The second alternative is desirable, for the first can lead only to increased hairsplitting and end in greater confusion than that which already exists.

Spickermann and Bremer's designation *Aspergillus ruber* (1902) is assigned to the series, because its members are predominantly producers of an intense red pigment and bear ascospores of the general size and pattern described by these authors. The only other described fungus possessing a similar ascospore and characterized by its red color is Bainier and Sartory's *Aspergillus sejunctus* (1911b).

Although it is now quite impossible to say what particular fungus either pair of investigators had at hand, both are believed to have worked with members of the large series now under consideration. *Aspergillus ruber* is retained since its description is more adequate for the series in addition to being the prior species.

To accentuate cultural similarities and differences between the strains studied, a wide variety of culture media has been employed. Based upon their appearance in culture, the strains fall into a few well-defined subgroups (see figs. 29 and 30). The strains belonging to one of these seem

to represent the fungus described by Speckermann and Bremer (1902) and are at the same time most abundant in the entire series hence are considered as typical of *A. ruber* (figs 29 A and 30 A). The general characters of the remaining subgroups are limited to how the extreme cultural variation

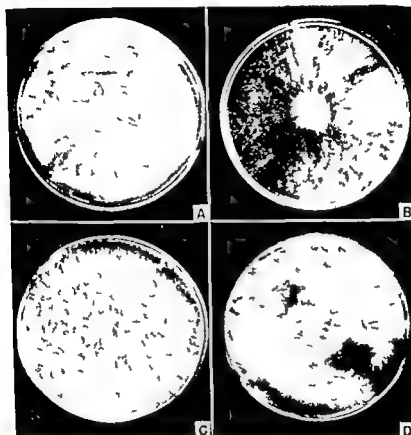


FIG 29 Different colony types developed in the *Aspergillus ruber* series in three weeks at room temperature upon 20 percent sucrose Czapek agar. A Typical *A. ruber* NRRL No 52. B NRRL No 70 characterized by thin colonies with mycelium largely submerged. C NRRL No 60 colony floccose bearing abundant perithecia and few conidial heads. and D NRRL No 75 deep floccose colony with very abundant conidial heads and only scattered perithecia.

that is to be expected within the series but specific names are withheld because to perpetuate or propose such would multiply rather than clarify the confused nomenclature of this abundant and variable series of organisms.

To illustrate more definitely the variation that occurs, the following outline of possible lines of separation is inserted

- A Colonies predominantly perithecial
- 1 Colonies red perithecia abundant in a layer at the agar surface and overgrown by a felt of red encrusted hyphae
A. ruber (Spieck. and Brem.) Thom and Church NRRL Nos 57-49
 - 2 Perithecia abundant in a loose floccose overgrowth of red hyphae as well as in a layer at the agar surface
 NRRL No 60
 - 3 Colonies thin orange red perithecia abundant in old cultures and on very concentrated media
 NRRL No 10
- B Colonies predominantly conidial
- 1 Colonies gray heads long stalked perithecia few
 NRRL No 75
- C Colonies mixed conidial and perithecial
- 1 Colonies orange red and green zonate perithecia borne at the agar surface and piled in a loose network of superficial hyphae
 NRRL No 71
- D Colonies predominantly floccose, colonies red brown perithecia and conidial heads few
A. locatensis Biourge⁴ NRRL No 76

Aspergillus ruber (Bremer)

Synonyms *A. ruber* (Spieckermann and Bremer) Thom and Church in *The Aspergilli* 112 1926

Eurotium rubrum Bremer in *Zeitschr f Untersuch d Nahrung und Genussmittel* IV 1901 p 72 also in *Die fett verzehr Organismen in Nahr u Futtermitteln* Dissert Munster 1902

E. rubrum Spieckermann and Bremer in *Landw Jahrb* 31 81-128 1902

A. sejunctus Bain and Sart *Soc Mycol de France Bul Trimest* 27 361-367 pl VI 1911

Colonies upon Czapek's solution agar (3 percent sucrose) more restricted plane (fig 24 C₁) orange brown to red brown in color perithecia generally abundant though often abortive conidial heads pea green to olive green abundant in some strains few and largely vestigial in others reverse orange red to maroon

Colonies upon Czapek's solution agar with 20 percent of sucrose spread ing rapidly and broadly in a regular manner or unevenly, plane predominantly red ranging from ferrugineous to morocco red perithecia very abundant borne in a dense layer at the agar surface and largely concealed within and beneath a close textured felt of red-encrusted hyphae conidial heads projecting above the felt pale gray green to deep olive gray more or less abundant and generally crowded near the center or scattered unevenly

Species name not recognized as valid by authors of this publication

over the colony (Pl III D figs 29 A and 30 A) reverse in shades of dark red brown

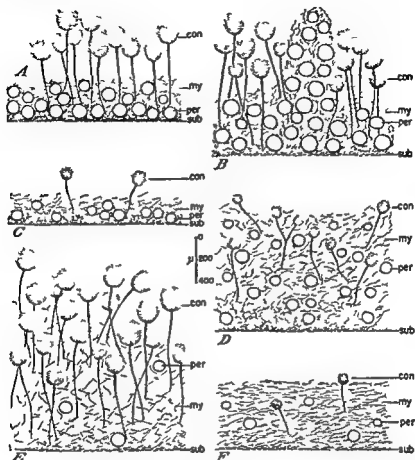


FIG 30 Diagrammatic representation of cross sections of different colony types in the *Aspergillus ruber* series developed at room temperature upon 20 percent sucrose Czapek agar showing relative abundance and disposition of the conidial heads (con) and perithecia (per) and the amount and character of the mycelium (my) above the substratum (sub) A Typical colony of *A. ruber* as seen in NRRL No 52 B F, atypical colonies as seen respectively in NRRL No 31 No 70 No 65 No 75 and No 76 Scale approximate (Reprinted from Thom and Raper *The Aspergillus glaucus* Group USDA Misc Pub 426 1-46 1941)

Perithecia very abundant, largely enmeshed in a felt at the agar surface (fig 30 A) yellow to orange red, spherical to subspherical, mostly 80 to 120 μ , though occasionally up to 140 μ in diameter; asci 12 to 15 μ ; ascospores lenticular, 5.2 to 6.0 μ by 4.4 to 4.8 μ , with furrow generally evident as a broad and shallow depression around the spore equator; ridges low and

often inconspicuous walls smooth except for minute roughness along equatorial ridges (fig 27 B). Conidial heads generally abundant, numerous in localized areas or scattered thinly over the colony pale blue green, radiate 150 to 250 μ in diameter conidiophore smooth, colorless to orange brown 500 to 750 μ in length, broadening to 14 to 16 μ where it passes into the subglobose vesicular area of 25 to 35 μ diameter, sterigmata in a single series 7 to 9 μ by 4 to 5 μ conidia elliptical to subglobose, closely spinulose, mostly 5 to 6.5 μ in long axis

Aspergillus ruber is represented in this study by NRRL Nos 52 53, and others Of 31 strains examined belonging to the whole series 19 showed colonies and microscopic characters that place them within the species *Aspergillus ruber* as described above Although the majority of strains belonging to the *A. ruber* series produce plane colonies as noted in the description occasionally strains may produce colonies more or less wrinkled

Culture NRRL No 65 (figs 29 C and 30 D) represents a subseries of several strains that differ from the above not only in colony character upon 20 percent sucrose Czapek agar as indicated in the preceding key, but also in their growth upon media of lower concentration These grow slowly and poorly on Czapek (3 percent sucrose) potato dextrose and wort agars, producing small raised colonies of 1 to 2 cm in diameter bearing neither normal conidial heads nor perithecia

Strain NRRL No 70 (figs 29 B and 30 C) produces abundant perithecia only on very dry areas of the substratum in old cultures or on media containing a sucrose concentration of 40 percent or more In contrast to other strains, this fungus grows better upon media containing 4 percent agar than the usual 1.2 percent agar further establishing its xerophytic character

Strains such as NRRL No 75 (figs 29 D and 30 E) occasionally encountered, are predominantly conidial and characterized by rampant hyphae bearing abundant conidial heads piled in floccose masses above the substratum and upon the edges of the culture dish or tube They thus produce colonies markedly in contrast with the usual *Aspergillus ruber* concept But the character of their ascospores together with the occurrence of occasional sectors in colonies of these strains showing the usual mixture of perithecia and conidial heads relates them definitely with *A. ruber*

Strain NRRL No 71 (fig 30 B) represents a subsection of the series in which conidial heads are abundant and generally arranged in fairly definite zones and patches with loose clusters of perithecia irregularly and conspicuously distributed among and above the grouped green heads These strains are further characterized by somewhat larger perithecia than those of NRRL No 52 being mostly in the range of 125 to 150 μ in diameter and by producing less red color in the colonies and in their reverse

Culture NRRL No 76 (fig 30 F) is characterized by a close felt of red brown hyphae which completely covers the agar surface and in which scattered perithecia are borne. Conidial heads are scarce and largely confined to the colony margin. This strain which was included in Gould and Raistrick's study of pigmentation in the *Aspergillus glaucus* group (1934) was received from George Smith under the name *Aspergillus loaisiensis* and attributed to Biourge. Except for its dark color this fungus in culture bears a striking resemblance to one received from Baarn as *Aspergillus profusus* (NRRL No 44) which showed similar floccose habits. However, the ascospores of the latter are smaller and less furrowed and are essentially smooth along the equatorial margin. The degree of relationship between the two is questionable.

In addition to the ascospore strains definitely placeable in the series George Smith has recently described *A. proliferans* in which the proliferation of the sterigmata has become so pronounced as to become the most conspicuous character while perithecium formation has been suppressed. In colony characters however it belongs here. This strain diverges further from the type as described but may be arbitrarily placed here by the branching of its simplified heads and the size and markings of its conidia.

Aspergillus proliferans George Smith in Brit Mycol Soc Trans 26(1/2)
26 Pl III 1943

Colonies on Czapek's solution agar spreading very slowly with growth at first largely submerged then with matted floccose aerial mycelium white changing to yellowish shades sporing tardily with conidial areas gray green reverse yellowish brown on wort agar growing slowly but better than on Czapek with mycelium white then yellow and finally orange and tardy development of gray green to gray conidial areas becoming more deeply floccose in age especially at shallow end of the slope reverse yellow normal conidial heads loosely radiate conidiophore smooth thin walled usually with one or two septa 4 to 14 μ in diameter vesicles occasionally almost globose more frequently obconical or mere broadening of the ends of the conidiophores up to about 20 μ in diameter sterigmata when normal in one series 8 to 11 μ by 3.5 to 6 μ often elongate septate and bearing small secondary heads frequently resembling heads of monoverticillate *Penicillia* or with upper portion much swollen and appearing almost as very large thick walled conidia with long connectives up to 20 μ in diameter with normal and swollen sterigmata often appearing in the same head conidia globose or subglobose rough fairly dark-colored 5 to 9.5 μ in diameter perithecia not found (Species description after George Smith)

Aspergillus halophilus Sartory Sartory and Meyer (in Ann Mycol 28(3/4) 367 363 Pl III 1930) appears from the description based upon colonies grown upon licorice sticks to have been some member of this general group. No cultures have been available for comparison hence placement near *A. proliferans* of George Smith can be only tentative. If placement were to be based upon their figure 1⁹ it might be a species of *Scopulariopsis*.

ASPERGILLUS CHEVALIERI SERIES

Ascospores lenticular, mostly 4.6 to 5.0 μ by 3.4 to 3.8 μ occasionally up to 5.2 μ in long axis with walls smooth or slightly rough with crests prominent flexuous often recurved and with furrow conspicuous but consisting more of a trough between extended equatorial crests than a depression in the spore wall.

Strains belonging to this series show appreciable difference in colony character and to a limited degree in the surface markings of their ascospores. The ascospores of all, however, are characterized by their continuous, prominent equatorial crests which do not form an integral part of the spore wall, but extend well beyond the margin of the spore body proper. To use Mangin's exceedingly descriptive term they are characteristically "pulley form."

The following key will serve to differentiate groups of strains within the series.

A Ascospore walls smooth

- 1 Crests prominent thin flexuous often recurved

A. chevalieri (Mangin) Thom and Church

- 2 Crests evident low usually erect

A. chevalieri var. *multiascosporus* Nakazawa et al¹¹

B Ascospore walls more or less roughened

- 1 Crests thin flexuous often recurved conidia roughened *A. oriolus* Bourgois

- 2 Crests thicker usually erect conidia smooth

A. chevalieri var. *intermedius* Thom and Raper

Aspergillus chevalieri (Mangin) Thom and Church The Aspergilli p. 11 1926

Synonym *Eurotium chevalieri* Mangin Ann des Sci Nat Bot (Ser 9) 10 361-362, fig. 12 1909

Colonies upon Czapek's solution agar (3 percent sucrose) restricted plane closely felted bluish gray in center with typical heads and perithecia largely confined to marginal area (fig. 24 C₁) reverse maroon in center to orange at margin.

Colonies upon Czapek's solution agar with 20 percent of sucrose growing best at 30° C. or above spreading plane to somewhat wrinkled in central

* Species name not recognized as valid by authors of this publication

area (fig. 31 A) with abundant conidial heads in blue green shades distributed evenly over the whole surface or more crowded in localized areas projecting above a layer of abundant perithecia embedded in orange red hyphae at the agar surface reverse in shades of orange red to brown more intense in center.

Perithecia abundant and closely embedded in a felt of orange red encrusted hyphae (fig. 26 B) mostly 100 to 140 μ occasionally up to 150 μ globose to subglobose yellow to orange ascig 9 to 10 μ ascopores lenticular 4.6 to 5.0 μ by 3.4 to 3.8 μ with walls smooth with equatorial crests prominent thin and often recurved and with furrow consisting more of a trough between parallel crests than an equatorial depression in the spore body



FIG. 31. Comparative growth of members of the *Aspergillus cheilarii* series in three weeks at room temperature upon 20 percent sucrose Czapek agar. A. Typical *A. cheilarii* (NRRL No. 78). B. *A. cheilarii* var. *intermedius* (NRRL No. 80).

(fig. 27 C). Conidial heads abundant pale blue green appearing radiate from divergent conidial chains mostly 125 to 175 μ in diameter occasionally larger conidiophores mostly 700 to 850 μ in length enlarging to a vesicular apex somewhat globose 25 to 35 μ in diameter sterigmata in a single series closely packed 5 to 7 μ by 3 to 3.5 μ conidia subglobose spinulose mostly 4.0 to 5.5 μ in diameter.

Aspergillus cheilarii is represented in the present study by cultures NRRL Nos. 78, 79 and others. The species name is limited to strains bearing ascopores with smooth walls and prominent thin equatorial crests because it is believed that the strains most nearly represent the organism described by Mangin (1909). When grown upon 20 percent sucrose Czapek agar these strains are further characterized by their predominantly orange

red colonies, pale blue green conidial heads and dark colored reverse. Within the series different strains vary in the quantity of conidial heads produced, e.g. NRRL No 79 regularly produces an abundance of heads, NRRL No 78 relatively few.

Strains of this series are not so commonly encountered as are those of the *A. repens*, *A. amstelodami*, or *A. ruber* series. And within the series strains that conform with the typical species description are relatively less numerous than in these other series. In the present study 14 strains belonging to the *A. chealieri* series have been examined and only 6 or less than half, are wholly representative of the species *A. chealieri*.

The series as a whole seems to be relatively unstable and certain strains and groups of strains appear transitional between this series and the *A. repens* series on the one hand and the *A. amstelodami* series on the other.

Culture NRRL No 88 received from Baarn as the type of *Aspergillus chealieri* var. *multiascosporus* Nakazawa, Takeda, Okada and Sino points toward the *A. repens* series. Its ascospores have smooth walls as in the typical strains of *A. chealieri*, but bear low erect crests in contrast to the thin flexuous crests characteristic of this species. Spores lacking crests are occasionally seen and these closely resemble *A. repens*. The colony upon 20 percent sucrose Czapek agar is definitely of the character of *A. chealieri*. Nakazawa and coworkers (1934) separated it from *A. chealieri* because of its more floccose habit and its more abundant production of perithecia. The former character is evident in the authors' cultures but perithecia are not produced more abundantly than in certain strains entirely typical of *A. chealieri*.

Another variation from the typical species is seen in culture NRRL No 87 received from George Smith as *Aspergillus oriolus* and attributed to Biourge. The ascospores of this culture (and another that is in the NRRL collection No 81) have crests typical of *A. chealieri* but the spore walls are finely roughened over their entire surfaces. This character is suggestive of *A. amstelodami* although the roughening of the wall is slight in comparison with that species. The colony upon 20 percent sucrose Czapek agar is essentially like that of typical strains of *A. chealieri* but is less red in color and bears fewer conidial heads. Although these cultures can be distinguished from type they are not recognized as warranting separation.

The roughening of the ascospore wall is further accentuated in a group of four apparently similar strains which it is believed are truly intermediate between *A. chealieri* and *A. amstelodami*. Because their ascospores bear crests of the *A. chealieri* type and hence appear pulley form they are retained in the species. However because they differ from typical strains in additional particulars they are considered a new variety, namely *Aspergillus chealieri* var. *intermedius*.

Aspergillus chevalieri (Mangin) var *intermedius* Thom and Raper in U S D A Misc. Publ. No. 426 p. 21 1941

Colonies upon Czapek's solution agar with 20 percent sucrose differing from the species in texture and color and presenting withal a picture intermediate between *A. chevalieri* and *A. amstelodami* (fig. 31 B). Ascospores lenticular mostly 4.6 to 5.2μ by 3.6 to 4.0μ occasionally 5.4μ in long axis with walls roughened and with prominent equatorial crests. Conidial heads dull green radiate to columnar mostly 100 to 120μ in diameter and up to 175μ in length conidia elliptical to subglobose smooth walled mostly 3 to 4μ in long axis.

Represented in this study by culture NRRL No. 52 which was received from George Smith as No. 107 and bore the following notation: Isolated G. S. from cotton yarn 1927. Close to *A. chevalieri*—differs in having smooth small conidia and ascospores somewhat larger than type. Duplicated by three additional strains received from European sources.

Aspergillus chevalieri var *intermedius* appears to be transitional between the *A. chevalieri* and the *A. amstelodami* series. Such a view is supported (1) by the pattern of the ascospores which shows both the extended and often recurved equatorial crests characteristic of *A. chevalieri* and the rough spore walls of *A. amstelodami*; and (2) by the coloration of the colony. *Aspergillus chevalieri* var *intermedius* upon 20 percent sucrose Czapek agar becomes orange yellow above and orange to light brown in reverse. *A. amstelodami* remains bright yellow with reverse uncolored whereas *A. chevalieri* becomes red in the colony and reverse. The smoothness of conidia in *A. chevalieri* var *intermedius* is a distinctive character and appears in neither *A. chevalieri* nor *A. amstelodami*. Although this variety from many points of view appears to be a hybrid proof of such origin is lacking.

Aspergillus diplocystis (Sartory Sartory Hufschmidt and Meyer) Dodge Med. Myc. p. 625 1935. Syn. *Eurotium diplocyste* Sartory, Sartory Hufschmidt and Meyer in Compt. Rend. Soc. Biol. 104: 881 883 1930. Not *E. diplocystis* H. and Br. Jour. Linn. Soc. 24: 55-56 Tab. 10 1875.

Characterization: Colonies greenish yellow becoming yellow from penithecia. Conidiophores erect 50 to 100μ high 3.1 to 3.7μ in diameter membrane thick hyaline. Sterigmata confined to a portion of head 5 to 6.5μ by 1.5 to 2.5μ . Secondary sterigmata small conidia spherical 2.5 to 3μ in diameter slightly ellipsoid green (tendre to cendre) sterigmata sometimes abortive and proliferous. Penithecia canary yellow asci 4 to 6μ by 5 to 7μ containing 8 ascospores which are ovoid with a furrow and two crests 1.5 to 2.5μ by 1.8 to 3μ .

This description suggests an *Aspergillus* with the heads approximating *A. nidulans* and the penithecia of *A. chevalieri*. Ascospore measurements as reported are appreciably smaller than those of *A. chevalieri* or any other known species of *Aspergillus*. It was described from a case of onychomycosis from the thumb and the great toe. Tentatively placed in the *A. chevalieri* series. The name is invalid because of *E. diplocystis* H. and Br. 1875.

ASPERGILLUS AMSTELODAMI SERIES

Ascospores 4.7 to 5 μ by 3 μ to 3.8 μ lenticular, colorless with equatorial furrow conspicuous, broadly V shaped and flanked by broad irregular ridges, with walls irregularly and unevenly ridged or roughened over the entire surface

Included in this series are strains that differ greatly in colony appearance. However, their close relationship is demonstrated by the similarity in size and pattern of their ascospores and is further shown by the dark olive green color of their conidial heads, the bright yellow color of their perithecia, and the absence of any red either in the colonies or their reverse.

The following key is designed to show the variation that occurs within the series and to offer a means of separating strains or groups of strains that are culturally distinct.

A Colonies predominantly perithecial

- 1 Conidial heads abundant in central area and often in concentric zones
A. amstelodami (Mangin) Thom and Church
- 2 Conidial heads widely scattered or lacking
NRRL No. 113

B Colonies predominantly conidial

- 1 Perithecia widely scattered superficial
NRRL No. 111
- 2 Perithecia abundant in a felted layer above the conidial heads
A. montevideensis Talice and MacKinnon

C Colonies very thin perithecia and conidial heads widely scattered

NRRL No. 110

Aspergillus amstelodami (Mangin) Thom and Church, The Aspergilli p. 113 1920

Synonyms *Eurotium amstelodami* Mangin in Ann. des Sci. Nat., Bot. (ser. 9) 10: 360-361 1909

E. repens var. *amstelodami* Vuill. Soc. Mycol. de France, Bul. Trimest. 36: 131 1920

Colonies upon Czapek's solution agar (3 percent sucrose) restricted 4 to 6 cm. in diameter, plane or closely wrinkled, yellow to dull yellow gray in color from abundant perithecia admixed with sterile hyphae and developing conidial heads; reverse uncolored, becoming tawny in age.

Colonies upon Czapek's solution agar with 20 percent of sucrose spreading 8 to 10 cm. in diameter, more or less wrinkled and zonate (Pl. III, F and fig. 32 A), perithecia very abundant and clustered in masses forming a dense layer at the agar surface (fig. 26 D), bright yellow in color, lending a characteristic appearance to the colony; conidial heads deep olive green, abundant in colony center and scattered more or less unevenly over the whole surface, occasionally obscuring the layer of perithecia beneath.

Reverse persistently yellow under perithecial areas more or less green where conidial areas predominate

Perithecia globose to subglobose mostly 115 to 140 μ in diameter occasionally up to 160 μ not covered by or embedded within a felt of sterile hyphae (fig 26 D) asci mostly 10 to 12 μ 8-spored ascospores lenticular,

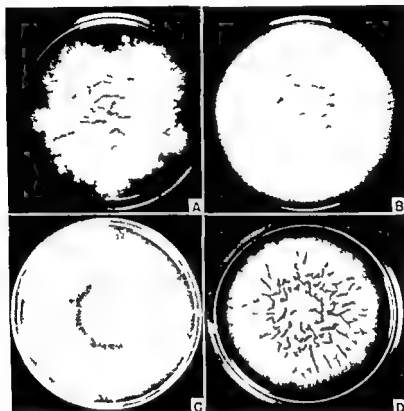


FIG 30 Comparative growth of members of the *Aspergillus amstelodami* series A Typical *A. amstelodami* NRRL No 90 B NRRL No 113 a strain producing abundant perithecia and few conidial heads C NRRL No 111 strain producing abundant conidial heads and very few perithecia D *A. montecidensis* NRRL No 108

4.7 to 5.0 μ by 3.6 to 3.8 μ with prominent V shaped equatorial furrow and broad irregular ridges and with walls roughened over their entire surfaces (fig 27 D) Conidial heads radiate-columnar mostly 120 to 150 μ in diameter occasionally larger conidiophores colorless to pale yellow green 275 to 350 μ in length broadening to 10 to 12 μ in diameter below the vesicle vesicle subglobose 18 to 25 μ in diameter sterigmata about 5 to

6.5 μ by 2.5 to 3.5 μ conidia finely spinulose subglobose, variable in size ranging from 3.5 to 5.2 μ mostly about 4 μ in long axis.

Represented in the NRRL collection by cultures Nos 89, 90 and many others.

Thirty two cultures belonging to this series have been examined in the present study. Included in this number are the authors' own isolates from a wide variety of sources together with cultures contributed by collaborators in this country and abroad. Of these more than three fourths regularly produce colonies conforming with the above description of the species *A. amstelodami*. Although wide variation in colony character does occur within the series, it is obvious that such variations are exceptional rather than commonplace. Accordingly, it is not believed advisable to assign or create specific or varietal names for these variations although they differ markedly from the typical *A. amstelodami* in gross appearance. An exception to this policy has been made in the case of cultures received as *A. montecidensis* for reasons that will be considered later.

As indicated in the preceding key to the series marked variation from the normal cultural character of *A. amstelodami* occurs along certain divergent lines.

Culture NRRL No 113 (fig 32 B) received from Baarn as *Eurotium repens* (Cda) DeBary and Wor var *amstelodami* Vuill (1920) represents a variation that tends toward an almost complete suppression of the conidial phase with only an occasional small and atypical head present.

In the opposite direction culture NRRL No 111 (fig 32 C) recently received from Bliss in California (isolated from date fruits) represents a variation that produces a dense stand of conidial heads and only occasional perithecia these being borne above rather than below the layer of crowded conidial heads.

In contrast to both of the preceding culture NRRL No 110 isolated from an old shoe produces an extremely thin spreading colony that bears only widely scattered perithecia or conidial heads.

A fourth distinct variation is represented by culture NRRL No 108 received in 1932 from Talce as *A. montecidensis* Talce and MacKinnon (1931). This fungus is characterized by an initially strong development of the conidial phase and subsequently of perithecia in a felted overgrowth which in the colony center more or less obscures the underlying conidial layer. Perithecia and conidial heads are somewhat smaller than in strains of *A. amstelodami*. Although this culture does not differ from *A. amstelodami* more widely than the variations previously noted since it has an imputed pathogenic history and since it has been described and distributed widely under the name *Aspergillus montecidensis* it is believed advisable to retain the name in association with this culture. Accordingly the writers include the following emended description.

Aspergillus montevideensis Talice and MacKinnon in Soc de Biol (Paris)
 Compt Rend 108 1007-1009 1931 : emend Thom and Raper
 U S Dept of Agr Misc Pub 426 p 26 1941

Colonies upon Czapek's solution agar (3 percent sucrose) restricted radiate-ulate with zonation evident toward the margin central area showing coremia perithecia few or lacking reverse and agar very dark almost black.

Colonies on Czapek's solution agar with 20 percent of sucrose spreading wrinkled and buckled (fig 32 D) at first bluish green from massed conidial head with central area later becoming yellow from developing perithecia in a more or less tufted overgrowth of somewhat floccose mycelium reverse in yellow green shades to deep olive in colony center.

Perithecia abundant of variable size and irregular shape with relatively few fertile asci and a copores late in developing commonly 75 to 100 μ in diameter occasionally larger asci 10 to 12 μ in diameter ascospores lenticular roughened with broad and prominent furrow flanked by low acute and irregular ridges mostly 4.8 to 5.2 μ by 3.6 to 4.0 μ occasional pores larger or smaller. Conidial heads very abundant small somewhat columnar with few conidial chains mostly 70 to 80 μ wide occasionally up to 100 μ conidiophore up to 300 to 350 μ long frequently very short when borne upon the aerial mycelium broadening to a hemispherical dome like vesicular area at the apex commonly deep green or greenish brown vesicle mostly 15 to 20 μ in diameter occasionally larger or smaller sterigmata in one series relatively short and thick 6 to 7 μ by 3 to 3.5 μ conidia roughened subglobose commonly 4 to 5 μ by 3 to 4 μ occasionally 5.5 μ diameter.

Type culture isolated by Talice and MacKinnon from the tympanic membrane of the human ear (1931). It is carried in the NRRL collection as No 108.

LARGE SPORED SPECIES OF THE HERBARIOPUS SERIES

Under *Eurotium herbariorum* Lk. Maving includes all of the members of the group with ascospores more than 6.6 μ in long axis (1909). In a general way this represents a very common usage in older literature beginning as far back as Corda in the 1830's. Because neither measurements nor markings of the ascospores were given no one can fix the type of *E. herbariorum*. In general the species in the large-spored group have both conidia and ascospores definitely larger than those in series already described. They become very conspicuous to the collector who finds the anomalous situation of an overabundance of published names and a dearth of isolations. Over a period of many years the scarcity of strains isolated in this laboratory which show ascospores larger than 7.0 μ leads the authors to believe that such forms are definitely rare if not abnormal. This obser-

vation is, in effect, confirmed by George Smith (1931) From textiles in particular he has isolated many small spored strains but none with large spores. Possibly the present collection contains as many large spored strains as it does because the authors have regarded them as curiosities, and for that reason retained them whereas scores of strains of such common species as *A. repens* or *A. amstelodami* have been isolated and forthwith discarded.

In contrast to the small spored forms where complete duplication between large numbers of isolates is the rule, among the large spored forms there is a marked tendency for each strain to present a somewhat different cultural picture, which is commonly coupled with differences in morphology. This would suggest that these forms are unstable and variable, but such a conclusion is refuted by their behavior in culture. To illustrate culture NRRL No. 131, a strain of *Aspergillus echinulatus*, has for 20 years of continuous culture by the authors retained its distinguishing characters. Similarly the single known strain of *A. medius* has been under observation in this and European laboratories for more than 40 years without appreciable change.

Thus the problem of assigning a relatively small number of quite distinct strains is presented. To describe each of them would merely add to the confusion already existing hence they have been grouped somewhat, choosing either historic cultures that have become widely distributed or cultures of marked individuality as representing specific names. Homogeneity among the strains brought together is not claimed. The names *A. glaucus* and *E. herbariorum* are not identified with particular organisms in this discussion.

In setting apart a so-called 'large spored series' the authors do not in any sense wish to imply close relationship or genetic continuity within this subgroup. Species are grouped together primarily as a matter of convenience, and (*E.*) *herbariorum* is selected as the series designation primarily because of Mangin's usage of this species name to cover all of the large spored forms.

Key

A Conidial heads green

- 1 Asci ripening within 2 to 4 weeks
 - a Ascospores 6.5 to 7.5 μ in long axis *A. manginii* comb
 - b Ascospores 7.5 to 8.5 μ in long axis *A. umbrinus* Bain and Bart
 - c Ascospores 9.0 to 10.0 μ in long axis
 - 1 *echinulatus* (Delacr.) Thom and Church
- 2 Asci ripening slowly 2 to 3 months colonies favored by 40 per cent sugar
 - a Ascospores with equatorial ridges and furrow *A. medius* Miss
 - b Ascospores usually without equatorial ridges and furrow
 - 1 *A. carnei* (Biourge) Thom and Raper

B Conidial heads white

A. niveo glaucus Thom and Raper

Aspergillus mangini (Mangin) n. comb.

Synonyms *Eurotium herbariorum* ser. *minor* Mangin Ann. des Sci. Nat. Bot. (ser. 9) 10: 365, 1909.

Aspergillus minor (Mangin) Thom and Raper, U. S. D. A. Misc. Publ. No. 426, p. 27, 1941.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, attaining a diameter of only 1 to 2 cm. in 3 weeks; irregular and wrinkled, cream colored to bluish brown; conidial heads present or lacking; small perithecia present or lacking; mostly abortive; reverse uncolored to orange-maroon.

Colonies upon Czapek's solution agar with 20 percent of sucrose plane or somewhat wrinkled in the central area, spreading evenly, attaining a diameter of 8 to 10 cm. in 3 weeks (fig. 33 A); predominantly brick red in color, becoming maroon in age; perithecia abundant and borne in a close felt of red-encrusted hyphae at the agar surface; conidial heads few in number, projecting above the perithecial layer, generally distributed over the entire colony but occasionally concentrated in localized areas; reverse in shades of deep red brown.

Perithecia abundant, largely embedded in and obscured by a close mycelial felt at the agar surface; yellow to orange, globose to subglobose, mostly 100 to 120 μ in diameter, occasionally up to 150 μ ; asci 14 to 16 μ ; ascospores lenticular, commonly 6.6 to 7.4 μ by 5.2 to 5.8 μ , occasionally up to 7.8 μ in long axis, finely roughened in the equatorial area, ridges low and rounded or pyramidal in section, furrow generally definite, shallow but often steep-sided, V-shaped.

Conidial heads few, generally scattered, projecting above the perithecial layer; pale blue-green in color, radiate, mostly 150 to 200 μ in diameter but frequently larger; conidiophores smooth, pale to dark brown, mostly 700 to 800 μ in length, occasionally reaching 1 mm, broadening to 15 to 18 μ below the vesicular apex; vesicles subglobose, 30 to 40 μ ; sterigmata in a single series, 8 to 10 μ by 4 to 5 μ ; conidia dull green, elliptical to subglobose, mostly 6.0 to 7.5 μ and frequently 8.0 μ in long axis.

Represented by culture NRRL No. 117, isolated from an unpainted board, as type, and by several additional cultures isolated in this laboratory. Culture NRRL No. 115, received in 1937 from Oscar W. Richards of the Spencer Lens Company, differs from the type by producing ascospores of somewhat smaller size and with less evident furrow and ridges. Thus it may represent a strain transitional between *Aspergillus mangini* and *A. ruber*. However, it is not sufficiently different from *A. mangini* either culturally or morphologically to warrant its description as a separate species or as a distinct variety.

Mangin (1909), in his study of the group, found specimens in his collec-



FIG 33 Comparative growth of large spored members of the *Aspergillus glaucus* group upon 20 percent sucrose Czapek agar at room temperature A *A. manginii* NRRL No 117 4 weeks B *A. umbrinus* NRRL No 170 4 weeks C *A. echinulatus* NRRL No 131 6 weeks D *A. nireoglaucus* NRRL No 177 4 weeks E *A. medius* NRRL No 125 6 weeks F *A. carneus* NRRL No 126 4 months (Reprinted from Thom and Raper *The Aspergillus glaucus* Group USDA Misc Pub 426 1-46 1941)

tion with ascospores less than 7.5μ in long axis yet larger than those of the small-spored forms which he described. Having no faith in any of the descriptions existing at the time, he called the aggregate *Eurotium herbariorum* series *minor*. The cultures cited above have sufficient common characters to warrant the belief that they are variants of a common stock which may be constituted a species aggregate to which we apply the name *Aspergillus mangini*.

Aspergillus umbrosus Bain and Sart. in Soc. Mycol. de France. Bul. Trimest. 28: 267-269 pl. VII. 1912.

Probable synonyms: 4. *mutabilis* Bain and Sart., in Soc. Mycol. de France. Bul. Trimest. 27: 458 pl. XVII. 1911.
A. mollis Bain and Sart. Soc. Mycol. de France. Bul. Trimest. 27: 453 pl. XVI. 1911.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted attaining a diameter of 0.5 to 1.0 cm. in 3 weeks raised tufted white to orange red bearing neither perithecia nor conidial heads reverse colorless to orange brown.

Colonies upon Czapek's solution agar with 20 percent of sucrose plane or somewhat wrinkled spreading mainly or irregularly (fig. 33 B) reaching a diameter of 8 to 10 cm. in 3 weeks predominantly vinaceous red to orange brown in color consisting largely of a surface felt of sterile hyphae encrusted with orange red granules enmeshing abundant perithecia occasionally characterized by a loose floccose overgrowth bearing scattered perithecia conidial heads pale blue green widely scattered and projecting above the perithecial layer reverse in red brown shades.

Perithecia abundant yellow to orange globose to subglobose largely embedded in a felt of sterile red-encrusted hyphae at the agar surface occasionally borne in a loose aerial felt mostly 120 to 140 μ in diameter rarely up to 175 μ ; asci 14 to 16 μ ; ascospores lenticular mostly 7.2 to 8.0 μ by 5.6 to 6.4 μ ; occasional spores up to 8.4 μ in long axis finely roughened to smooth in the equatorial areas ridges low and generally rounded furrow shallow commonly V shaped (fig. 31 A) conidial heads few scattered projecting above the perithecial layer pale bluish green radiate compact mostly 175 to 250 μ in diameter conidiophores smooth colorless to brownish 700 to 850 μ in length broadening to 15 to 20 μ below the expanded domelike vesicular apex vesicle 25 to 40 μ in diameter sterigmata in a single series 10 to 12 μ by 4.5 to 6 μ ; conidia pale green elliptical to subglobose spinulose mostly 7 to 8 μ in long axis frequently larger.

Represented in the present collection by culture NRRL No. 120 received in 1925 from Dr. Florence A. McCormick by European strains and by several cultures isolated by the authors.

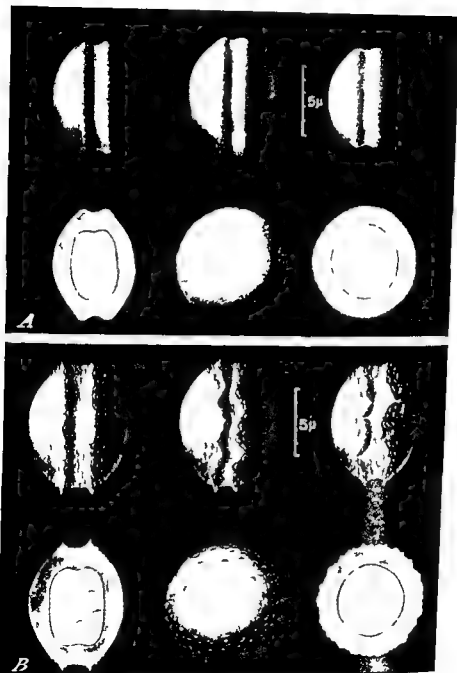


FIG 34. Ascomyces representative of *A. umbrosus* and *A. echinulatus*. In each species upper left, right, and center spores represent surface profile views; lower left, optical section in profile; lower center, surface in face view; and lower right, optical section in face view. (Reprinted from Thom and Harper, *The Aspergillus glaucus* Group, U S D A Misc Pub 426, 1-46, 1941.)

Culture NRRL No 123 contributed by Dr Paul Simonart as an unnamed culture from the Biourge collection differs from the species as above described by consistently producing ascospores with walls entirely smooth whereas in other characters the ascospores duplicate essentially those of NRRL No 120. Further NRRL No 123 produces colonies of lighter color than other strains under observation and may in fact represent a fungus comparable to that described as *Aspergillus mutabilis* by Bainier and Sartory.

Aspergillus umbrosus, *A. mutabilis* and *A. mollis* were described by Bainier and Sartory (1911c, 1912b) primarily upon the basis of colony color (pigment production) and conidial apparatus with the ascospores of *A. umbrosus* recorded as slightly less in long axis (8.0 by 5.6 μ) than those of the other species (8.4 by 5.6 μ). After careful consideration of the three descriptions and detailed study of the strains in the authors' possession showing in general the ascospore described by Bainier and Sartory, it is believed that they had at hand three cultural variants of the same species. *A. umbrosus* is retained as the species designation as it is believed that their description of this species more adequately pictures the cultural and morphological characters of the fungi under consideration than either of the earlier descriptions which are left as probable synonyms.

Aspergillus echinulatus (Delaer) Thom and Church. The Aspergilli
p 107 1920

Synonyms *Eurotium echinulatum* Delacr. Soc. Mycol. de France. Bul. Trimest. 9, 266 pl. XIV fig. III 1893.

A. brunneus Delacr. in Bul. Soc. Mycol. France 9, 185 Pl. XI fig. 9 1893, was described as the conidial stage.

E. ferruculosum Vuill. Soc. Mycol. de France. Bul. Trimest. 34, 83 1918.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, 1.5 to 3.0 cm. in diameter after 4 weeks, marginal area blue green from conidial heads and central portion reddish brown from an overgrowth of sterile encrusted hyphae; perithecia lacking; reverse deep orange.

Colonies upon Czapek's solution agar with 20 percent of sucrose slow growing, plane or somewhat wrinkled, spreading irregularly, attaining a diameter of 7 to 8 cm. in 4 weeks (fig. 33 C), commonly mottled in appearance due to the uneven distribution of green conidial heads above the underlying orange red perithecial layer; conidial heads bottle green, abundant, commonly crowded in localized areas but scattered thinly throughout the remainder of the colony; perithecia abundant and borne in a felt of hyphae encrusted with red granules at the agar surface, conspicuous where not obscured by massed green heads; reverse cinnamon to deep red brown.

Perthecia abundant, embedded in a loose felt of sterile red hyphae on the agar surface (fig 26 E) yellow, globose to subglobose mostly 125 to 150 μ in diameter, and occasionally up to 175 μ , asci 18 to 22 μ ascospores lenticular, mostly 9 to 10 μ by 6.5 to 7.5 μ occasionally up to 11 μ in long axis conspicuously roughened in the equatorial area, furrow pronounced broad, ridges prominent and irregular (fig. 34 B)

Conidial heads densely crowded in localized areas and scattered throughout the remainder of the colony, bottle green in color, radiate consisting of relatively few, long divergent chains of conidia commonly 250 to 300 μ in diameter but often larger or smaller, conidiophores smooth walled colorless to brown shades commonly 700 to 850 μ in length, occasionally in excess of 1 mm, broadening from 5 to 7 μ at the base to 15 to 20 μ below the vesicular apex, vesicle 25 to 35 μ in diameter, consisting of a domelike terminus of the broadening conidiophore sterigmata in a single series, not crowded bottle shaped 12 to 15 μ by 5 to 7 μ , conidia elliptical pyriform, or subglobose echinulate, mostly 8 to 10 μ in long axis commonly larger or smaller extremely variable

Represented by NRRL No 131 isolated in 1921 from figs received from California. A subculture of this strain forwarded by Miss Margaret Church about 1926, is maintained in the Centraalbureau, the two lines remain identical. No ascospore stage has in the authors experience been found in culture. NRRL No 133 received in 1937 from George Smith as *A. echinulatus* Delac and obtained by him from Biourge but its conidial development duplicates NRRL No 131 and it is apparently correctly as signed. Da Fonseca's and the Centraalbureau's isolations of *A. echinulatus* maintained at Baarn produce somewhat smaller ascospores (8 to 9 μ by 6.2 to 7.0 μ) and conidia than No 131 but otherwise agree essentially with the species description as given above. Somewhat further reduction in ascospore size is seen in cultures NRRL No 523 isolated from honey, and NRRL No 137 received from George Smith and Raistrick as *Aspergillus mongolicus* Biourge (nomen nudum). Although these are less red in color than No 131 and appear distinct in culture the authors do not feel warranted in separating them as a species or variety believing that they represent only variations from the general type designated as *A. echinulatus*.

Bainier and Sartory described 4 *disjunctus* (1911b) and *A. repandus* (1911c) as vigorous species possessing ascospores 11 by 6 μ and 11.2 by 5.6 μ respectively. Authentic cultures of these species are not now available but the descriptions as published would seem to place them close to *A. echinulatus*.

Probable Synonyms

Several *Aspergilli* with ascospores ranging near that described for *A. echinulatus* appear in the literature. Unfortunately the details of a co

spore markings are not given and there is a dearth of data to identify them. Some of the names are given.

A. disjunctus Bainier and Sartory in Soc. Mycol. France Bul. 27: 346-368. Pl. XI, 1911. Ascospores described as 11.2 by 5.6 μ with furrow and crests.

A. repandus Bainier and Sartory in Soc. Mycol. France Bul. 27: 463. Pl. XVIII, 1911. Ascospores described as 11 by 6 μ with furrow but no crests.

A. menciensis Sartory and Flament in Compt. Rend. Soc. Biol. (Paris) 83: 1114-1115, 1900. Ascospores 10 by 4.7 μ with furrow and crests.

A. godfrini Sartory and Roederer in Ann. Française pour l'Avancement des Sciences, 4th Session, Tunis, 1913, pp. 601-663, 1914. Conidial stage only. This was growing at blood heat and warmer. Its general description suggests affinity with the large spored species of the *A. glaucus* group. It has not been seen in culture by us.

Strains with the particular measurements reported for the three ascosporic species listed above have not come into our collection, yet presumably they and many more with minor variations may be found.

Aspergillus medius Meiss in Bot. Ztg. 55: (337)-344 (353)-357, 1897.

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted, 0.5 to 1.5 cm. diameter in 6 weeks, tufted, consisting of a dense growth of yellow-brown hyphae bearing neither conidial heads nor perithecia, reverse in shades of yellow-brown.

Colonies upon Czapek's solution agar with 20 percent of sucrose at room temperature very slow growing (optimum 20 C \pm) strongly wrinkled, tufted, irregular in outline, attaining a diameter of 5 to 6 cm. in 6 weeks (fig. 33 E); colony center deep orange-red becoming yellow to white at the margin, which is characterized by bundles (becoming branching columns at lower temperatures) of hyphae bearing dark green conidial heads in the manner of loose divergent coremia, perithecia ripening very slowly, maturing ascospores in 2 to 3 months, mostly abortive, conidial heads relatively few (more abundant and larger at lower temperatures) and borne either in coremiform masses or scattered throughout the colony, reverse in shades of orange-maroon.

Perithecia scattered, mostly abortive, borne in a dense felt of orange-red hyphae, very slowly ripening, globose to very irregular in form, extremely variable in size, rarely attaining a diameter of 125 μ , containing very few mature ascospores, asci 18 to 20 μ , ascospores sparingly produced, lenticular, mostly 8.8 to 9.6 μ by 6.0 to 6.8 μ , occasionally 10 μ in long axis, somewhat roughened in the equatorial region, furrow broad and shallow, ridges prominent, relatively thin and irregular.

Conidial heads deep green, radiate, compact, and of two types. Small heads 100 to 150 μ in diameter, borne on loose coremiform columns, and larger heads 200 to 250 μ in diameter, often scattered throughout the colony.

produced more abundantly at 12° to 15° C than at room temperature conidiophores colorless to brown mostly 250 to 350 μ in length enlarging to 15 to 20 μ below the vesicle, vesicle subglobose mostly 30 to 40 μ in diameter, sterigmata in a single series, crowded short 7 to 8 μ by 4 to 5 μ , conidia green, globose to subglobose, finely echinulate, thick walled mostly 8 to 10 μ in diameter, but frequently larger or smaller

Represented in the NRRL collection by culture No 124 which was received in 1924 from Raistrick, who in turn received it from the Centraal bureau It is believed to be Meissner's original strain (1897) Subcultures of this strain are currently maintained at Baarn and by George Smith in London The three lines remain identical as shown by parallel cultures during recent study

This fungus is distinguished particularly by (1) its very slow growth upon 20 percent sucrose Czapek agar at room temperature (2) its tardiness in producing perithecia and especially in ripening ascospores (3) its sparse production of ascospores and (4) its formation of aerial hyphal bundles bearing conidial heads in loose coremiform fashion Further it grows much more rapidly upon Czapek agar containing 40 percent of sucrose than upon that containing 20 percent a difference in concentration which does not materially affect the growth rate of such vigorous species as *A. repens* and *A. chevalieri* Growth is much more rapid at 20° C than at 28 to 30° C (fig 12) The fungus attains a more favorable form at the lower temperature at which there is a heavier growth of mycelium a more extensive development of aerial hyphal columns and a greater production of conidial heads and perithecia

Culturally this fungus is easily separated from all other species of the group, except possibly *A. carnei*:

Aspergillus carnei: (Biourge) Thom and Rapet, U S Dept Agr Misc Pub 426 p 34 1941

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted reaching a diameter of only 3 to 4 mm in 6 to 8 weeks thin white bearing neither conidial heads nor perithecia reverse colorless

Colonies upon Czapek's solution agar with 20 percent of sucrose at room temperature extremely slow growing (optimum 18 to 20 °C) reaching a diameter of 7 to 8 cm in 6 to 8 weeks irregular in outline somewhat floccose forming a deep felt bearing abundant perithecia and scattered conidial heads (fig 33 F), orange brown in central area to orange at margin from abundant perithecia in a loose network of sterile hyphae encrusted with orange red granules reverse in orange red shades

Perithecia late in developing abundant yellow to orange globose to subglobose mostly 125 to 175 μ in diameter but frequently larger or smaller borne in a loose floccose felt of sterile brown hyphae asci 16 to 18 μ

typically 8-spored frequently with some or all spores aborted ascospores lenticular variable in size and pattern mostly 7.2 to 8.4μ by 6 to 11.5μ but often larger (up to 9.0μ in long axis) or smaller (down to 6.5μ in long axis), generally smooth walled but occasionally roughened in equatorial area, generally rounded but often flattened and occasionally indented with ridges wholly absent or indefinite, and with furrow absent or present as a trace only

Conidial heads sparsely produced commonly scattered dull gray green radiate compact mostly 150 to 200μ but often up to 250μ in diameter, conidiophores smooth walled colorless long commonly up to 2 mm in length uniform in diameter 12 to 18μ to just below the vesicle vesicle subglobose 40 to 50μ in diameter and occasionally larger sterigmata in a single series crowded bottle shaped 10 to 12μ by 5 to 6μ conidia globose to subglobose echinulate dull green mostly 8 to 10μ

Species description based upon culture NRRL No. 126 received in 1937 as *Aspergillus carnyi* Biourge from George Smith and by him earlier from Biourge. Presumably the culture is type although Biourge's description of the species remains unpublished. The culture is distinct, not only differing in its colony character and in the length of its conidiophores but especially in the variable character of its ascospores. The majority of spores although much larger resemble those of *A. repens* whereas spores with rough walls are occasionally produced. This culture is therefore somewhat of an exception to the general rule of constancy in ascospore pattern and the variability of its spores affords one of the best characters for its identification.

Aspergillus nneo-glaucus Thom and Raper U. S. Dept. Agr. Misc. Pub.
426 p. 35 1941

Synonyms *A. glaucus* mut. *alba* Bloch Deut. Bot. Gesell. Ber. 50
248-256 1932

A. glaucus var. *albida* Speg. An. del Mus. Nac. de Buenos
Aires 6 332 1899

Colonies upon Czapek's solution agar (3 percent sucrose) very restricted 1 cm. in diameter after 4 weeks white to cream bearing abundant small conidial heads but no perithecia reverse colorless to yellow brown

Colonies upon Czapek's solution agar with 20 percent of sucrose slow growing plane spreading irregularly 6 to 8 cm. in diameter after 4 weeks (fig. 33 D) thinning toward the margin with mycelium in shades of yellow orange becoming cinnamon brown in age more or less obscured by abundant white heads and with perithecia abundant yellow (Pl. III F) embedded and irregularly clustered in a close felt at the agar surface reverse yellow at margin to deep brown at colony center

Perithecia abundant yellow globose to subglobose mostly 100 to 125μ

in diameter occasionally larger commonly clustered borne in an interrupted surface felt of buff to brown hyphae asci 15 to 17 μ in diameter ascospores lenticular, mostly 7.2 to 7.8 μ by 5.0 to 5.6 μ smooth walled

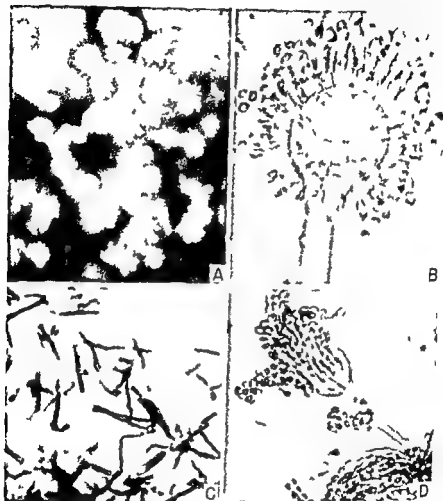


FIG 35 *Aspergillus glaucus* group conidial heads A and B A *niteo glaucus* NRRL No 127 A surface view showing loose radiate character of heads $\times 35$ B photomicrograph of the same showing large globose vesicle fertile over almost the entire surface $\times 600$ C and D *Aspergillus restrictus* NRRL No 154 C surface view showing long columnar heads $\times 120$ D photomicrograph of the same showing characteristic small vesicle fertile on the uppermost surface only $\times 500$

except in equatorial area furrows broad and shallow ridges prominent roughened rounded or acute and often appearing ragged Conidial heads abundant white (fig 35 A) often becoming browned in age radiate mostly 250 to 300 μ in diameter conidiophores smooth walled colorless to brown,

mostly 1 000 to 1 500 μ , rarely longer broadening to 16 to 20 μ below the vesicle vesicle subglobose 40 to 50 μ in diameter (fig 35 B) sterigmata in a single series crowded 8 to 10 μ by 3 to 4 μ conidia elliptical to pyriform colorless spinulose 6 to 8 μ in long axis

The cultural description is based upon NRRL No 127 of this collection as type, this is duplicated by NRRL No 130 received from Baarn in 1938 as *Aspergillus glaucus* Link mut *alba* Bloch Culture NRRL No 128 received in 1935 from George Smith and Raistrick and by them from Biourge as *A. albidus* Speg. differs from type by consistently producing a greater quantity of conidial heads which are commonly of smaller size the asexual stage of the two is indistinguishable Apparently the name 'albidus' is a manuscript use in which Spegazzini's variety (1899) has been raised to species rank presumably by Biourge Blochwitz mutation *albus* assigned to *A. glaucus* is untenable because no definite organism can be designated as *A. glaucus* Furthermore the specific name *albus* applied to an *Aspergillus* has already been used in another section of the genus

It is possible that Blochwitz (1932c) is right in regarding this as a mutation but there is nothing to indicate which particular large-spored form is the parent species The strain maintains its identity in culture and hence must be regarded as a species Tuill (1939) in contrast has described white mutants of *A. nidulans* and *A. fumigatus* and has properly designated them as mutants for they appeared in cultures under observation and are known to have been derived from typical chromogenic strains

THE ASPERGILLUS RESTRICTUS SERIES

Thom and Church (1926) called this series the Intermediate Forms between the *A. glaucus* and the *A. fumigatus* groups George Smith wishing to emphasize the resemblance of the conidial apparatus to the monoverrucillate *Penicillia* called the lot the *A. penicilloides* group (1931) thus suggesting Spegazzini's species as the typical member However the important relationship indicated by the structure of the conidial apparatus is not with *Penicillium* but with the *A. glaucus* group of which these forms appear to be merely reduced members All of these forms like many of the *A. glaucus* forms grow characteristically under conditions of physiological drought—represented by their frequency upon mildewed textiles as studied by Smith (1928) and Galloway (1930) or in concentrated cane products as reported by Owen (1923) Similarly we have found them in many situations in which physiological drought is attained by physical dryness or osmotic concentration attained by the presence of high percentage of sugar or sodium chloride

This natural relationship is on the whole better indicated by accepting Smith's *A. restrictus* as typical and regarding the other known members

of this series as allied species. To perpetuate an assignment to a subdivision entitled the *Microaspergilli*, as suggested by some authors would complicate nomenclature without compensating values. The series is therefore keyed as a part of the great *A. glaucus* group.

Series diagnosis. *Penthetecia* not found. Colonies growing weakly or restrictedly upon Czapek's solution agar, more freely upon wort agar, especially well on high concentrations of sugar or salt, green dark green grayish green to brownish green in various strains and under varying conditions. Surface growth consisting of conidiophores only or of mycelial felts more or less buckled or heaped, conidiophores smooth slender more or less sinuous, septate, vesicles vary from convex or lens like areas on the broadened apices of conidiophores to definitely ovate to globose enlargements, fertile over all or the upper fraction of such surfaces. Heads mostly definitely columnar, less commonly radiate hemispherical or almost globose, especially when young. Sterigmata in one series, mostly closely packed over the fertile area, varying from 2 to 3 μ by 5 to 6 μ up to 3 to 4 μ by 6 to 10 μ . Conidia barrel shaped to ovate mostly in dark greenish shades, smooth or slowly becoming echinulate or roughened as in the *A. glaucus* group commonly adherent into long chains which are packed into columns.

Series Key

- A Conidiophores broadening upward to produce a convex vesicular apex varying from 8 to 20 μ in diameter producing a long slender column of conidia. *A. gracilis* Bainier
- B Conidiophores broadening more abruptly forming a more definite vesicular area.
 - 1 Vesicles more convex toward hemispherical.
 - a Slime development evident. *A. conicus* Bloch
 - b Slime absent or not conspicuous. *A. restrictus* G. Smith
 - 2 Vesicles ovate to hemispherical. Columns of conidial chains more or less conspicuous. *A. penicilloides* Speg.

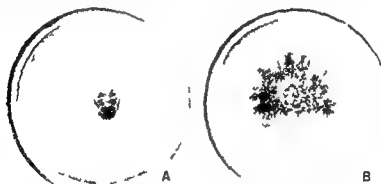
Differences between the above may appear to be of somewhat minor character. Nevertheless, each of the sections accounts for sufficient literature to necessitate separate consideration.

Aspergillus gracilis Bainier in Bul. Soc. Myc. France. 23: 92 pl. 1 \ figs 11-14. 1907.

Colonies on Czapek's solution agar very slow growing reaching a diameter of only a few millimeters in several weeks (fig. 36A) variously plane or convoluted or buckled with close textured mycelium at first white then slowly green to very dark green with radiating lines of vegetative mycelium about the denser area of the colony growing somewhat better upon wort agar and upon Czapek's agar containing high concentrations of sugar reverse in yellowish shades. Conidial heads in columns up to 200 or 300 μ long.

by 10 to 20 even 25μ in diameter straight or twisted Conidiophores mostly arise as very short branches of aerial hyphae up to 20 to 30μ long or less commonly up to 100 to 120μ even up to 200μ and gradually broadening toward the apex to a vesicular area which is very flat dome like almost the effect of a truncated cone 8 10 to 20μ or more in diameter bearing a single series of sterigmata 6 8 10 by 2 to 3μ or in particular strains growing out into little conidiophores producing secondary heads conidia at first barrel form then subglobose about 3μ in long axis in Bainier's strain progressively larger in related strains No perithecia found

The type culture has not been seen Forms with approximately the morphology described however have appeared in strains NRRL No 145 (Thom 4246) from moldy corn Thom 4197.3 (culture lost) from Owen in



10 36 A *Aspergillus gracilis* NRRL No 145 on Czapek's solution agar with 50 percent sucrose after incubation for 2 weeks at room temperature B *A. restrictus* NRRL No 154 on Czapek's solution agar with 20 percent sucrose after 18 days at room temperature

the sugar laboratory New Orleans and a strain from Thaxter appearing as a contaminant in a *Papulaspora* culture *A. gracilis* may thus be assumed to represent a form occasionally found especially in very concentrated substrata Biourge later contributed a culture as type of *A. hypoxanthinus* originally described by him as *Penicillium hypoxanthinum* Biourge (1923) Three other cultures from Biourge labeled P (*Microaspergillus*) *hickeyi*, *Microaspergillus albo-marginatus* and P (U) *guegueni* (figured in his monograph Plate XX but not described) appear in culture to be only minor variations of this general form Later Biourge sent his undescribed *A. sartoryi* This grew more freely and showed the conidiophore and vesicle of *A. gracilis* conidia were 6 to 7μ or greater in long axis definitely rough and corresponded almost exactly with a culture received from George Smith

as *A. gracilis* (NRRL No 156) Whether further accumulation of strains will justify giving Biourge's proposed name *A. sartoryi*, sectional or varietal status or merely emphasize the completeness of the series as showing great variations in structural detail is left uncertain. There does not appear to be any warrant for preserving *A. gracilis* var *exiguus* Bainier and Sartory (1912 a). According to the description this variety differs slightly in physiological characters from *A. gracilis* Bainier.

A. conicus Blochwitz in Dale Ann Mycol 12 38 1914. Previously described by Dale as a *Penicillium* in Ann Mycol 10 465 1912.

See also Thom and Church 'The Aspergilli' p 120 1920.

The outstanding character of this species is found by microscopic examination of colonies which become buckled or contorted from a close felting of mycelia. Relationship back toward the *A. glaucus* group is found in the sterigmata and in the elliptical conidia. Heads differing little in microscopic structure from *A. gracilis* are found to be more or less completely submerged in dark green to almost black slime. Many strains have been collected from widely separated regions showing all gradations from a trace of slime only to complete submergence of the heads particularly in old cultures.

Dale isolated the strain first described from English soil and sent duplicate cultures labeled *Penicillium* sp. to Blochwitz and to Thom. Thom returned the very brief descriptive note without name as published by Dale in 1912. Later Blochwitz proposed by letter to her the name only, *A. conicus* which was published by Dale in 1914 without further description. Later in his own publication *Die Gattung Aspergillus* (1929) Blochwitz denies the slime development as a character of his organism and redescribes the species in terms to make *A. conicus* cover the section of this group represented by Smith's *A. restrictus* (1931). Since the name was already in the literature for the slimy series which certainly appears in culture from widely separated places it should stand as originally applied. Whether the slime disappears in some strains long kept on artificial media is not settled.

In 1925 Biourge contributed under the manuscript name *A. cyanogenes* a strain which reproduced the characters given by Thom and Church for *A. conicus*. This or a nearly related organism appeared as a contaminant in several of the cultures received from Biourge. The culture (Thom No 4733 138) figured in his Monograph (1923) as No 126 in Plate XXI under *P. glabrum* Dale was another. Still another strain from Biourge labeled *A. viridans* differed only in the delayed development of the slimy covering of the columnar mass of conidia.

Veill in his study of the Aspergilli of New Zealand (1939) applied the name *A. caesiellus* Saito (1904) to what was manifestly some strain near *A. conicus* Blochwitz and then placed the whole group in that species

A. restrictus G. Smith in Jour. Text. Inst. 22 T115 fig. V 1931

Species characterization by George Smith

Colonies growing very poorly on Czapek agar growing moderately well on wort agar dark dull green gradually turning grey or brownish grey reverse in some cultures uncoloured in others green to dark green surface velvety at first, becoming wrinkled and often acquiring a warted appearance (Pl. IV V and fig. 36 B) heads forming long compact slender columns (fig. 35 C) up to 300μ by 20 to 30μ in diameter conidiophores arising mostly from substratum but also as branches of aerial hyphae commonly 50 to 100μ occasionally 150-200 μ long by 3 to 3.5μ in diameter often with one or two septa smooth sinuous, uncoloured vesicles flask shaped 7.5 to 14μ in diameter sterigmata in one series borne on upper surface of vesicles only 0 to 9μ by 2.5 to 3μ (fig. 28 C) conidia rough spinulose elliptical or somewhat pyriform often showing a distinct connective dark greenish brown 4 to 6.5μ by 3 to 4μ mostly 4.5 to 6μ by 3 to 3.5μ perithecia not found The young conidia are hyaline and cylindrical and almost appear to be segments of enormously elongated septate sterigmata They gradually swell without increasing in length at the same time becoming pigmented but even in old heads they adhere strongly together in columns of parallel chains and mounts (fig. 35 D) made in lactophenol usually show compact twisted columnar masses of ripe conidia both attached to and separated from the heads

It is evident from Smith's discussion of a large accumulation of cultures especially from the textile industry that the usual colony type in his experience is not the conicous type with its slime but the columnar head described as *A. restrictus* and accepted here as giving the most appropriate name to the series

Among organisms belonging to this series Smith isolated a single strain which differed from typical *A. restrictus* in showing somewhat larger dimensions throughout and more particularly in the production of conidia up to 10μ or more in length and to which he assigned the designation *Aspergillus restrictus* var. *B* (G. Smith Jour. Text. Inst. 22 T115 Figs IV VI and VIII 1931) Upon examination by us this culture (NRRL No. 148) was found to correspond fairly closely to his published description. However we do not believe it is sufficiently distinct to warrant continued separation as a variety since other strains showing intermediate dimensions are encountered

Aspergillus penicilloides Spegazzini, in Rev Agrar Veter
La Plata, p 246 1896

Spegazzini's description was emended by Thom and Church (The Aspergilli p 126, 1926) then broadened by Smith (Jour Text Inst 22, pp 114-115, 1931) as follows

'Colonies growing fairly slowly on wort agar rich dark green with paler edge, turning darker and duller, and finally becoming dirty greenish grey overgrown with sterile hyphae, reverse brown, greenish brown and dark green in patches, surface much wrinkled and folded heads globose when young 40 to 70 μ in diameter becoming columnar, somewhat ragged, and up to 200 μ long conidiophores arising either from substratum or from asexual hyphae, smooth, thin walled, 75 to 150 μ long by 6 to 10 μ in diameter, vesicles rather sharply marked off from conidiophores, pear shaped to subglobose 15 to 23 μ in diameter, fertile over the upper half or two thirds sterigmata in one series, crowded, 8 to 10 μ by 2.5 to 3.5 μ conidia ovate, barrel shaped or nearly spherical usually showing connective rough 3.5 to 5 μ by 3.2 to 4 μ with very dark colored walls

Thom and Church had strain No 4197 3 isolated from cane products in Louisiana by Owen and agreed to by Spegazzini, NRRL No 151 (Thom No 7) also from cane products fits this description satisfactorily as did also Bourges strain labeled *A. pertardus* Smith reports various strains from mildewed textiles

It would thus appear that the vesicular area in the series varies from the curved apex of a clavate conidiophore as in *A. gracilis* to a fairly well defined hemispherical vesicle as in *A. restrictus* and finally to an almost globose body as in *A. penicilloides*. All have so much in common with each other and with the *A. glaucus* group that their relationship as degraded mutants appears probable

Aspergillus itaconicus Kinoshita in Botan Mag Tokyo 45 60-61 1931

Probable synonym *A. varians* Wehmer in Bot Centralb 80 460-1
1899, also in Wehmer Monogr 77-79 Taf 1, fig
1 1899-1901

Diagnosis from Kinoshita's organism obtained from Dr Westerdijk Colonies on Czapek's solution agar forming dense felts 1 to 2 mm deep, white or yellowish ridged and irregular with scattered long-stalked green heads upon dry areas and on the glass, fruiting more abundantly upon malt agar and particularly upon Czapek's solution agar containing 20 percent sugar reverse of colony and agar yellow to orange reddish upon some media heads large light green globose to radiate, breaking up easily under the coverglass conidiophores smooth colorless 8 to 16 μ or larger in diameter and up to several millimeters in length under some conditions with

walls 0.5 to 1.5 μ in thickness and splitting lengthwise as *A. niger* when broken vesicles 15 to 40 μ , globose or subglobose (fig 28 D), stringy in series 8 to 9 μ by 1.5 to 2 μ conidia more or less pyriform, 4.3 to 5 μ by 3.5 to 4 μ finely echinulate of the *A. glaucus* type

Diagnosis is drawn from culture NRPL No 161 (No 5314 of Thom) received from Westerdijk as the type of Kinoshita's *A. itaconicus* and agrees closely with the description given by Wehmer for *A. varians* (cf Thom and Church The Aspergilli p 127 1926) not with Thom and Church's description of their culture No 115 which was subsequently lost *A. itaconicus* is so closely related in its physiological responses and in several of its structural characters to the *A. glaucus* group that it is placed as an extreme variant at the end of this whole group

Kinoshita described his organism as a producer of itaconic acid (1931a) and detailed his experimental work (1931b) The culture is distributed to laboratories outside of Japan seems to conform to Kinoshita's description and to produce the acid but in quantities too small for commercial development More recently Calam Oxford and Rastriek (1939) have recovered itaconic acid as a metabolic product of *A. terreus* From an industrial point of view this source appears to be far more promising

VARIATION IN THE ASPERGILLUS GLAUCUS GROUP

The genetic history of the Aspergilli is an untouched field Separation into large groups is easily made definite enough to include all but a few strains Within these groups variation is so great that differentiation of species requires critical examination and comparison of material including extensive culture Unwilling to undertake this Neill (1939) disposed of the whole group with yellow perithecia by calling them all *A. glaucus* On the other hand Mangin (1909), with the same problem of variability before him found the ascospores sufficiently distinctive and dependable to warrant proposing to separate *A. amstelodami* and *A. chailerti* as separate species in the *A. glaucus* group leaving certain aggregates admittedly inadequately studied Barnier and Sartory (1911b 1911c 1912) working with members of these ill-defined species used color production as the basis for separation They cited ascospore measurements as incidental details in description Because they appear to have had only a few strains in culture and to have described them all as new species the task presented few difficulties to them but unfortunately without the original cultures no one has ever been able to identify their species with confidence Rastriek and his colleagues (1934 1937 1938 1939) using cultures named as received and including an unpublished series from Biourge have given quantitative figures as to pigment production and pigment mixtures for each culture listed by the name on the tube without reporting comparative study of the morphology found

It is clear that wide mycelial or colony variations may be found in nature between strains that retain the ascospore characters of the species. Several such groups have been held by the authors for 30 years or more and furnish convincing evidence that Mangin was justified in using the ascospore as the stable and readily determinable integrating character. Some of these forms retain colony characters in fairly stable form through many transfers on many laboratory substrata and over a period of years. Others grown in various substrata in Petri-dish cultures show sectors or other irregularities in colony habit, which can be picked out and established as strain variants that maintain their special characteristics in continuous culture.

The possibility that variants of similar nature might be induced by chemical stimulation led Thom and Steinberg (1939) to select from the authors' collection certain strains that had remained fairly constant for many years. Of this group they subjected strain NRRL No 90 of *A. amstelodami* found apparently stable for 30 years to extensive chemical stimulation (1940a). These experiments yielded two groups of effects: (1) A progressive reduction in the production of conidial heads and of perithecia and (2) a great increase in the mass of vegetative mycelia. In no case was spore production completely suppressed although reduced to inconspicuous quantity. The conidia and ascospores when examined were found to have retained the size and markings characteristic for the species whereas the mass of vegetative mycelium became excessive and formed a floccose or cottony mass entirely different in colony appearance from the original.

At this point they reversed the procedure and applied stimulants designed to reestablish spore production (1940b). As a result, the final cultures show abundant green heads with normal conidia and numerous perithecia with ascospores retaining the characters of the species. In routine laboratory examination these extreme variants would not suggest the original strain of *A. amstelodami* although both types of variants produce conidia and ascospores typical of the species.

In 1928, Barnes studied the possibility of heat in inducing variations. He used a strain reported as *Eurotium herbariorum* (Wigg.) Link. which had been isolated and maintained in his laboratory for several years without apparent changes. Unfortunately no description of his normal strain was given but a strain received from Westerdijk as Barnes' normal strain proves to be identical with *A. amstelodami* (Baarn strain NRRL No 89 or strain No 90 as used by Thom and Steinberg). No reasons were offered for the original identification.

Barnes described a series of experiments in which the spores of his organism were subjected to heat under varied conditions then planted. From the resulting colonies he described 11 variants 6 of which are available in the Centraalbureau. The authors' transfers of these have been

checked against the descriptions in Barnes' paper and obviously represent his isolations.

In the following list his designations appear as quotations, followed by our identifications based upon careful cultural study.

Flame variant is *A. ruber*

Green flame = *A. repens*

Blue conidial = 1 *chalcidii* var. *intermedius*

Creamy is a pale yellowish strain of *Y* uilli genus *Cladosarum*

D Brown is *A. ustus*

C Yellow is 1 *amstelodami*

Of these C Yellow (NRRL No 112) shows the ascospore pattern and differs little from Barnes' normal strain or the authors' *A. amstelodami*.

Flame (NRRL No 59) Green flame (culture discarded) and Blue conidial (NRRL No 85) show ascospores of the *glaucus* group but differ markedly in pattern. Among large numbers of induced variations changes in the characters of the ascospore have not been found during this study.

D Brown (culture discarded) produces no ascospores but develops the hülle cells and conidial heads characteristic of the *Aspergillus ustus* group. These four forms belong to ubiquitous species quite abundant as contaminants where plant material is handled. Such contamination is not satisfactorily excluded by the work reported.

Creamy (NRRL No 143) presents a different problem. The possibility that this *Cladosarum* was actually derived from the normal *A. amstelodami* is not excluded. Proliferation of the sterigmata in the head of *Aspergilli*, especially among the *A. glaucus* lot, is very common. The branches produced sometimes are found sterile but usually become diminutive conidiophores with very small vesicles and groups of sterigmata producing normal spores. The *Y* uilli *Cladosarum olivaceum* (NRRL No 374) was found as a conspicuous variant or contaminant in their culture of *A. niger* (1938). The colony conidiophore vesicle primary sterigmata and initial secondary sterigmata are produced as in *A. niger* then instead of chains of conidia with the newest or youngest conidia at the bases of the chains and connected directly with the sterigmata, chains of cells are produced that replicate the sterigmata, occasionally a chain = interrupted by one cell producing a group of new chains, thus acting as a primary sterigma.

These chains of cells lengthen not at the base as in *Aspergillus* but at the distal end. In spite of prolonged search which shows that the cells toward the outer ends of such chains lack definiteness as sterigmata, the authors cannot confirm the finding of a single terminal conidium on each chain as reported by the *Y* uilli. This morphological picture is repeated by Barnes.

Creamy strain which must therefore be interpreted in terms of *Y* uilli genus. Only the two isolations are known thus far. Their failure to pro-

CHAPTER X

THE ASPERGILLUS FUMIGATUS GROUP

Outstanding Characters

Conidial heads columnar in shades of green through dark green to fuliginous

Vesicles flask shaped, typically fertile over the upper half

Sterigmata in one series crowded

Conidiophores smooth walled usually colored in shades of green

Conidia globose echinulate green

Working with lung material from birds dying of aspergillosis Fresenius, about 1850 described and figured the species *Aspergillus fumigatus* so well that there has never been any doubt as to the morphology of the mold present. The investigator of molds in culture however quickly finds that this type of conidiophore and head characterizes not a single pathogenic strain but a multitude of variant forms that are abundant in soil upon decaying vegetation, and in fact wherever organic materials are undergoing even the slightest aerobic decomposition. To all of these forms collectively the designation *Aspergillus fumigatus* group is applied.

The group falls naturally into two series

Cultures strictly conidial varying from velvety to floccose	(<i>fumigatus</i> series
Cultures producing perithecia and ascospores conidial development generally limited	(<i>fischeri</i> series

THE ASPERGILLUS FUMIGATUS SERIES

Aspergillus fumigatus Fresenius in *Beitrag zur Mykologie* p 81, pl 10, figs 1-11 Frankfurt, 1850-53 Thom and Church
The *Aspergilli* p 129 1926

Colonies upon Czapek's solution agar spreading broadly over the substratum in some strains strictly velvety (Pl IV B and fig 37 A) in others more or less floccose with varying amounts of tufted aerial mycelium to deep felted or extremely floccose forms (fig 37 B), white at first, becoming green with the development of heads but varying considerably in the final shade of green often becoming dark green to almost black in age. Reverse and substratum in some strains uncolored in others showing varying amounts of yellow or again passing over in dark red shades in age. Conidial heads columnar compact varying in measurement from strain to strain up to 400 by 50 μ but usually much shorter occasionally very small. Conidiophores

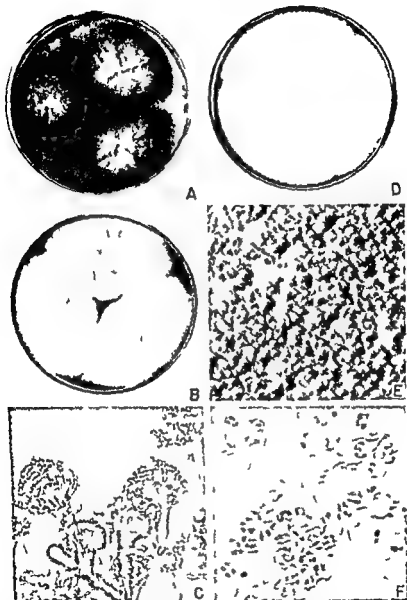


FIG 37 *Aspergillus fumigatus* group. A-C, *A. fumigatus*. A Typical heavy sporing strain NRRL No 178 on Czapek's solution agar 10 days room temperature. B Floccose light sporing strain of same species NRRL No 171 and C Photomicrograph of typical conidial heads showing characteristic form of vesicles and crowded sterigmata in a single series $\times 500$. D-F *Aspergillus fischeri*. D Strain NRRL No 186 growing upon Czapek's solution agar characterized by very abundant perithecia and few conidial heads. E Portion of a colony enlarged showing crowded perithecia more or less obscured by loose enveloping hyphae $\times 60$ and F Asci containing ascospores at various stages of maturity $\times 500$.

short smooth, usually densely crowded up to 300μ (in occasional strains up to 500μ) in length by 2 to 8μ in diameter frequently more or less green colored, especially in the upper part, arising directly from submerged hyphae or as very short branches from aerial hyphae septate or unseptate gradually enlarging upward and passing almost imperceptibly into the apical flask shaped vesicles. Vesicles up to 20 to 30μ in diameter usually fertile on the upper half only (fig 37 C). Sterigmata in one series usually about 11 to 8μ (varying from 5 to 10μ) by 2 to 3μ crowded closely packed with axes roughly parallel to the axis of the conidiophore. Conidia dark green in mass echinulate globose mostly 2.5 to 3μ in diameter with extremes ranging from 2 to 3.5μ . Sclerotia or perithecia are not found. The species grows well at temperatures up to 45°C or even higher, and is commonly present in compost and other material undergoing decomposition at high temperatures.

The species description presented is a composite rather than an exact citation of detailed data about one strain but NRRL No 163 (Thom No 118) may be considered typical. Organisms coming within this series as described are world wide in distribution and omnivorous in habit. They are regularly abundant in soil and in decomposing organic masses; they are recoverable from apparently sound cereals corn oats wheat etc., they are encountered as pathogens in the air passages of birds and occasionally as lung parasites of mammals including man. One strain (NRRL No 104) was named *A. cellulosa* by Hopfie (1919) because of its ability to break down cellulose but when examined it presented no morphologic differences from hundreds of common isolates from soil.

Efforts to induce perithecium formation by the conidial strains of *A. fumigatus* have been disappointing. Organisms maintained in culture over long periods and subjected to multitudes of transfers upon all sorts of substrata have failed to give any response suggesting perithecium formation.

Extremes of variation in different strains range from colonies characterized by crowded conidiophores rising vertically from submerged hyphae to a height of 300μ or perhaps at times 500μ then producing columns of conidia sometimes up to 400 by 50μ to very floccose forms in which spore formation is generally retarded and in which conidiophores develop as very short branches of aerial hyphae and produce short columnar heads. Many of these variants can be isolated and maintained in culture thus they have been made the types of species by earlier workers. Others encountered upon unique substrata have been given specific names in the belief that the substratum relation was obligate. Vuill (1939) isolated a buff-colored mutant from a typical green strain and applied to it the designation *A. fumigatus* var. *helvola* (fig 17 C). Shortly thereafter Steimberg and Thom (1940) likewise recovered an essentially uncolored mutant from a typical green strain. Both forms still remain unchanged in culture.

If the taxonomist could seize a half dozen widely spaced variants and destroy those which bridge the gaps between identification as separate species would be easy. This however becomes impossible when large numbers of isolates are cultivated and studied in comparative culture. In attempting to divide this great series into tangible entities earlier workers have created a long list of species and while we do not consider these to be valid they are presented in alphabetical order with the places of description. It is believed that all of these should be regarded as synonyms of *A. fumigatus* Fres.

- A. ariarius* Peck in N. Y. State Museum Rept 44 p. 20 pl. 4 figs 9-12 1891
A. bronchialis Blumentritt in Ber. Deutsch. Bot. Ges. 19 417-418 11 22 figs 1-6 1901 also ibid 23 419-427 Pl. 19 figs 1 3 6 7 8 19 22 1903
A. calyptratus Oudemans in Arch. Neerl. Ser. II 7 283 Tab. XIII 1907
A. cellulosa Hopffe in Centralb. f. Bakt. etc. Abt. 83 531-537 1919
A. deaseyi Spegazzini in Physis (Rev. Soc. Argentina Cien. Nat.) XIII 115-117 1 figure 1925 review only seen Rev. Appd. Mycol. 4 512 1925
A. fumigatus var. *alpha* Sion and Alexandrescu in Compt. Rend. Soc. Biol. (Paris) 64 288-289 1908
A. fumigatus var. *minimus* Sartory in Bul. Acad. Med. Paris 3 Ser. 82 301 305 1919
A. fumigatus var. *tumescens* Blumentritt in Ber. Deut. Bot. Ges. 23 410-427 Pl. 19 figs 5 6 18-21 1903
A. glaucoides Spring in Bull. Acad. Sci. Belg. 19 560-562 1852
A. lignerensis Cost. et Lucet in Ann. Sci. Nat. Ser. 9 II 119 tab. 5 fig. 19-23 1905
A. nigrescens Robin in Histoire Naturelle des Végétaux Parasites p. 518 Paris 1853
A. pulmonum hominis Welcker in Kuchlenmeisters Parasiten II p. 144. This is discussed and figured by Theodor von Dusch in Virchow's Archiv (n. f. 1) 11 561-566 1857 but no ground is given for separating it from *A. fumigatus*.
A. ramosus Halber in Zeitschr. Parasit. 2 768-769 pl. 8 figs 1-6 1890
A. syncephalis Guerguen in Les Champignons parasites de l'homme et des animaux 209 pp. 12 pl. Paris 1904
A. virido-griseus Cost. and Lucet in Ann. Sci. Nat. Bot. IX 2 140 1903

THE ASPERGILLUS FISCHERI SERIES

Aspergillus fischeri Wehmer in Centralb. f. Bakt. etc. 2 abt. 18 390-2 figs 1-5 1907

Synonyms *A. fumigatus*—a cosmetic see Thom and Church in Am. Jour. Bot. 8 91-92 1918. See also Thom and Church The Aspergilli p. 132 1926

Sartorya fumigata Vuillemin in Compt. Rend. Acad. Sci. (Paris) 184, No. 3 136-137 1927

The conidial form is strikingly similar to *Aspergillus fumigatus*. Colonies grow well upon Czapek's solution agar with conidial heads sparingly pro-

duced at room temperature (fig 37 D) but more abundantly at 37° C. Conidial heads frequently small and generally of a lighter green color than those of typical *A. fumigatus*. Perithecia quickly and abundantly produced in most strains dominating the colony appearance (fig 37 E) commonly up to 300 μ in diameter, not colored or very pale salmon with walls scarcely colored consisting of a single layer of cells crushing easily covered by a loose network of uncolored sterile hyphae. Asci abundant,

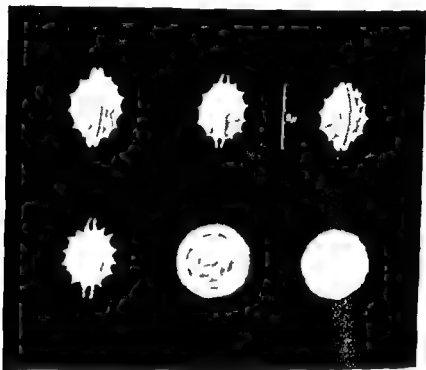


FIG 38 Ascospores of *Aspergillus fischeri* NRRL No 181 (= Thom No 4651 2). Upper left center and right represent surface profile views lower left optical section in profile lower center surface in face view and lower right optical section in face view.

8 spored filling the perithecium within a few days 8 to 10 μ by 10 to 12 μ subglobose (fig 37 F) breaking down quickly to leave the perithecium full of ripe ascospores. Ascospores biconvex uncolored usually about 7 by 4 μ consisting of a central body 5 by 4 μ with two frilled equatorial bands about 1 μ in width roughened with echinulations or anastomosing bands on each convex surface (fig 38) separating into two valves in germination.

Culture NRRL No 181 (Thom No 4651 2) received as type from Wehmer in 1923 is apparently identical with numerous isolations from American sources. Many strains have been seen including a series from sputum of human cases showing lung involvement by X ray examination.

It is regarded as world wide in distribution but seemingly not abundant anywhere. The species grows well at temperatures of 37° C and higher.

Some additional perithecial forms have been described with characters suggesting relationship with *A. fischeri*. However these are inadequately known in culture and assignment here must remain somewhat provisional.

A. malignus Lindt in Arch. Exp. Path. Pharmacol. 25: 271 fig. 111. 1889.

Lindt probably had before him some strain which corresponded closely with Wehmer's *Aspergillus fischeri* despite the fact that his description of the conidial apparatus offers sufficient contrast as to lead to question.

A. fumigatoides Bainier and Sartory in Bull. Soc. Myc. France 25: 112 pl. 5. 1909.

While the describers believed this strain close to *A. fumigatus* Thom and Raper (1941) found it necessary to place the organism studied under this name by Gould and Rastriek (1934) in *A. pseudoglaucus* although the original description and figures of Bainier and Sartory probably represented material close to or identical with *A. fischeri*.

Sartorya fumigata (Fres.) Vuille in Compt. Rend. Acad. Sci. (Paris) 184(3): 16-137. 1907.

Sartory, Sartory and Meyer (1906) reported that a culture of *A. fumigatus* subjected to radiation produced an ascomorphic form. Later this was designated by Vuillemin (1907) as a new genus *Sartorya*. Neither Vuillemin nor Sartory, Sartory and Meyer appear to have known the relation between *A. fumigatus* and *A. fischeri* although it was pointed out by Thom and Church in 1918. Since the material named *Sartorya* does not appear to have been distributed or fully described *Sartorya fumigata* (Fres.) Vuillemin may be regarded as a synonym for *A. fischeri* Wehmer and the generic name dropped.

It is believed probable that *Aspergillus fischeri* Wehmer represents the primary organism of this group and that from it the conidial form *A. fumigatus* developed as a species lacking entirely the ascomorphic phase. The fact that *A. fumigatus* was described first merely reflects the much greater abundance of this species.

Occurrence and Economic Importance

Aspergillus fumigatus is an extremely cosmopolitan mold and occurs with particular frequency in soil containing appreciable organic material, upon vegetable matter undergoing slow decomposition and upon imperfectly dried stored grains. The mold is an important agent in many decomposition processes, particularly at temperatures above 37° C. Growing successfully at 40° to 50° C. within the lower reaches of thermophilic decomposition it is able to operate within a range where most fungi are excluded. Whereas some forms have been described as very active agents of decomposition (e.g. *A. cellulosa* of Hopfle 1919) their more significant role is believed that of forerunners of active bacterial decomposition on the one hand and as slow destroyers of more resistant tissues on the other. *Aspergillus fischeri* though much less abundant may be found in situations generally similar to those yielding *A. fumigatus*.

Pathogenesis

There is an extensive pathological literature which covers the occurrence of *A. fumigatus* in lesions of birds and mammals including man together with biochemical and animal experimentation. Such experiments have repeatedly proved that the organism is pathogenic to fowls confined in congested quarters in which moldy grain, straw, and other plant remains are abundant. Direct inoculation to the cornea in laboratory animals causes lesions characteristic for the species.

Infection of human beings occasionally appears, and observations seem to indicate that the patients generally have been exposed to air carrying large numbers of spores. Allergists have reported asthmatic conditions arising from sensitization to this species and Bernton (1930) reports having successfully treated a patient by means of an extract prepared from the spores and mycelium of *A. fumigatus*. The occurrence of *A. fischeri* in cases grouped with *A. fumigatus* is clear indication that the pathogenic principle whatever it is, is generally present in the group although it may vary in its intensity among different strains as indicated by workers such as Costantin and Lucet (1905).

Among organisms known to be or believed to have been pathogenic strains of *A. fumigatus* the following named forms may be cited *A. gratioli*, *A. malignus*, *A. fumigatoides*, *A. virido-griseus*, *A. bronchialis*, *A. glaucoides*, *A. nigrescens*, *A. pulmonum hominis*, *A. ramosus* and *A. aurarius* (see p 151). For a more complete discussion of this group in relation to disease in birds and mammals the reader is referred to Thom and Church's *The Aspergilli* (1926) and Dodge's *Medical Mycology* (1935).

Antibiosis

Anslow and Raistrick (1938a) reported the production by *Aspergillus fumigatus* of a substance to which they applied the name *fumigatin* and in the same year (1938b) reported the species to produce a second metabolic product termed *spinulosin* which they had previously isolated from *Penicillium spinulosum*. In 1942 fumigatin was further discussed by Oxford and Raistrick as a powerful agent against such bacteria as *Bacillus anthracis*, *Escherichia coli*, *Salmonella typhi*, *murinum*, *Staphylococcus albus*, *S. aureus*, *Streptococcus viridans* and *Vibrio cholerae*. Also in 1942 Waksman, Horning and Spencer reported an antibiotic substance termed *fumigacin* to be produced by *A. fumigatus* and presented methods of differentiating this from fumigatin as studied by Raistrick and associates. Fumigacin was found to be both bactericidal and bacteriostatic in its action and to be effective in fairly high dilutions in inhibiting the growth of gram positive cocci and bacilli; it was much less effective against the gram negative members of the coli aerogenes group. Both fumigatin and fumigacin have been found toxic to experimental animals.

CHAPTER VI

THE *ASPERGILLUS NIDULANS* GROUP¹

Outstanding Characters

Conidial heads short columnar usually dark green with primary and secondary sterigmata

Conidiophores smooth walled more or less browned usually sinuate commonly less than 200 μ long and terminating in dome like or hemispherical vesicles

Conidia globose echinulate 3 to 4 μ in diameter

Perithecia usually present ascospores purple red in color and characterized by equatorial bands

Large thick walled globose bodies termed hülle cells (by Eidam), forming an irregular layer around the perithecia

Aspergillus (*Sterigmatocystis*) *nidulans* was described by Eidam in 1883. Since that time the general type of organism covered by his diagnosis has become fairly well known and certain striking characters have become recognized as defining a number of cosmopolitan strains or species commonly referred to as constituting the *Aspergillus nidulans* group.

The hülle cells of Eidam first described in *A. nidulans* appear also in various transformations in the *A. terreicolor*, *A. ustus* and *A. flavipes* groups together with other common characters indicative of close relationship. They do not appear in the *A. clavatus*, *A. glaucus* or *A. fumigatus* groups which are characterized by single sterigmata nor do they occur in the sclerotium forming groups *A. candidus*, *A. niger*, *A. wentii*, *A. tamaris*, *A. flavus* and *A. ochraceus*.

Group Key

I Ascospores present

A Ascospores smooth walled

1 Equatorial ridges two in number

a Ridges 0.5 to 1.0 μ wide margin entire

Conidial heads green

A. nidulans (Eidam) Went

Conidial heads white

A. nidulans mut. *alba* Yuill

b Ridges 1.5 to 1.8 μ wide margin entire

A. nidulans var. *latus* Thom and Raper

c Ridges 3.0 to 4.9 μ wide margin dissected starlike

A. terreicolor (Berk. and Br.) Thom and Raper

2 Equatorial ridges usually four in number

A. quadrilineatus Thom and Raper

II Ascospores rough walled

A. rugulosus Thom and Raper

Abridged from Thom and Raper: The *Aspergillus nidulans* group. Mycologia Vol. XXXI No. 6 653-669 Nov-Dec 1939

II Ascospores lacking

A Hülle cells forming irregular masses suggestive of sclerotia

A. caespitosus Raper and Thom

B Hülle cells absent, heavy walled sterile hyphae present

A. unguis (Emile Weil and Gaudin) Thom and Raper*Aspergillus nidulans* (Eidam) Wint in Rab. Krypt. Fl. 1: 62 1884Synonyms *Sterigmatocystis nidulans* Eidam in Cohn, Beitr. Biol. Pflanzen 3: 392-411 pl. 20-22 1883*Diplostephanus nidulans* (Eidam) Langeron, Compt. Rend. Soc. Biol. Paris, 87: 343-345 1922

Colonies upon Czapek's solution agar plane spreading broadly, dark cress green (Ridgway, Pl. XXXI) from abundant conidial heads during the first two weeks (Pl. IA IV C, and fig. 41 A) perithecia developing from the center of the colony outward after the first few days, separately produced often abundant (Pl. IV C) sectoring occasional, reverse of colony in varying shades of purplish red during the growing period becoming very dark in age. Heads short columnar ranging from 40 to 80 μ by 25 to 40 μ , commonly 60 to 70 μ by 30 to 35 μ (Pl. IB and fig. 4 C), conidiophores commonly sinuous with walls smooth in shades of cinnamon brown (Pl. IC) ranging from 60 to 130 μ , commonly 75 to 100 μ in length about 2.5 to 3 μ near the foot increasing to 3.5 to 5 μ below the hemispherical vesicle (fig. 39 A) vesicle 8 to 10 μ in diameter, sterigmata in two series primary 5 to 6 μ by 2 to 3 μ and secondary 5 to 6 μ by 2 to 2.5 μ conidia globose, rugulose 3 to 3.5 μ in diameter, green in mass.

Perithecia developed separately within or upon the conidial layer (Pl. IA) globose ranging from 100 to 175 μ in diameter commonly 125 to 140 μ with outer layer a yellowish to cinnamon colored envelope of scattered hyphae bearing hülle cells up to 25 μ in diameter wall composed of one layer of cells dark reddish purple in ripening becoming a mass of 8 spored asci which break down quickly leaving the ascospores free. Ascospores purple red lentikular smooth walled with 2 equatorial crests (Pl. ID and fig. 43 A) spore bodies about 3.8 to 4.5 μ in length by 3.5 to 4 μ in breadth equatorial crests pleated with margin sinuous and entire ranging from 0.5 to 1 μ in width (Table I).

Diagnosis based primarily upon culture NRRL No. 187 (Thom No. 46405) obtained from the Barmier collection in Paris. Other strains assigned to Eidam's species included many isolations from American soil and decaying vegetation as well as cultures from European contributors. Common.

The range of ascospore measurements found in a representative group of cultures is shown in the accompanying table.

In assigning Eidam's species name to the members of this series it is obvious that there are discrepancies. He described the perithecium as

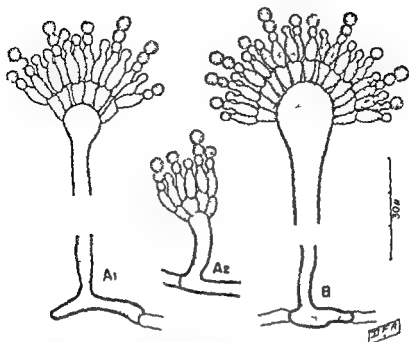


FIG 39 Conidial structure in the *Aspergillus nidulans* group $\times 900$ A₁ Typical conidial head of *A. nidulans* \RRL No 187 A₂ Diminutive head of the same strain B Typical head of *A. caespitosus* \RRL No 1979

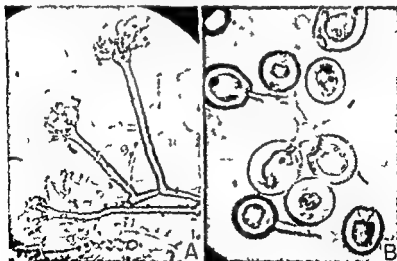


FIG 40 *Aspergillus nidulans* A Conidial heads $\times 370$ Conidiophores arise either from the substratum or from aerial hyphae B Hülle cells $\times 740$

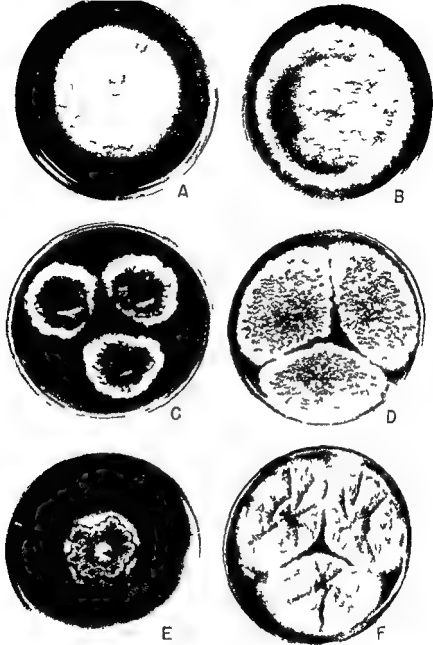


FIG. 41. *Aspergillus nidulans* group different species growing upon Czapek's solution agar at room temperature. A *A. nidulans* NRRL No 194 typical strain producing very abundant dark green conidial heads. B Naturally occurring mutation characterized by white heads isolated from the preceding strain by Edward Yuill and described as *A. nidulans* mut. alba. C *A. rugulosus* NRRL No 207, characterized by heavy perithecia production, an almost complete absence of conidial structures, and a tendency of colonies to split in central areas as shown. D *A. variegatus* NRRL No 1951 characterized by the production of abundant large perithecia. E *A. unguis* NRRL No 216 characterized by an absence of perithecia and hülle cells. F *A. caespitosus* NRRL No 1929 characterized by the production of masses of hülle cells suggesting abortive perithecia.

having a firm almost sclerotoid wall whereas the wall is found to contain but one layer of cells. He figured the asci as few and scattered in a mycelial matrix within which ascospore production occupied many weeks. One isolated strain in our collection produces asci in this manner. For it the varietal name *A. nidulans* var *latus* was proposed by Thom and Raper (1939) on account of very broad crests on the ascospore in contrast to the usual types in *A. nidulans* which come much more closely to the σ indicated in Eadams figures.

TABLE I
Ascospore variation in strains of Aspergillus nidulans

Cult. number	Over Eadams' mean of spores	Width of crests	Diameter of spore bodies
NRRL 193	6.2-6.6 x 3.6-3.8 μ	1.0 μ \pm	4.2-4.6 x 3.6-3.8 μ
NRRL 188	6.0-6.6 x 3.6-3.9 μ	1.0 μ \pm	4.0-4.6 x 3.6-3.9 μ
NRRL 189	6.0-6.4 x 3.6-3.8 μ	1.0 μ \pm	4.0-4.4 x 3.6-3.8 μ
NRRL 194	5.4-5.8 x 3.6-3.8 μ	0.6-0.8 μ	4.0-4.4 x 3.6-3.8 μ
NRRL 195	5.4-5.8 x 3.6-3.8 μ	0.6-0.8 μ	4.0-4.4 x 3.6-3.8 μ
NRRL 191	5.4-5.8 x 3.6-3.9 μ	0.6-0.8 μ	4.0-4.4 x 3.6-3.9 μ
NRRL 187	5.4-5.8 x 3.6-3.8 μ	0.7-0.8 μ	3.9-4.3 x 3.6-3.8 μ
NRRL 192	5.0-5.4 x 3.6-3.8 μ	0.5-0.6 μ	4.0-4.4 x 3.6-3.8 μ
NRRL 198	4.8-5.4 x 3.6-3.8 μ	0.5-0.6 μ	3.8-4.4 x 3.6-3.8 μ
NRRL 190	4.8-5.2 x 3.6-3.8 μ	0.5 μ \pm	3.8-4.2 x 3.6-3.8 μ

White mutant from culture NRRL No. 191 described by Yull as *Aspergillus nidulans* mut. *alba*

A. nidulans mut. *alba* Yull in Jour. of Botany (London)
170 pl. 618 1939

Colonies of the variety on Czapek's solution agar differ from the species in the entire absence of green color (fig. 41 B). The ascospores have the characters of the species.

Culture obtained by Yull as a mutant from a normal green strain of *A. nidulans* under investigation in his laboratory. Our record number is NRRL 195; the normal and parent strain is NRRL 191.

Aspergillus nidulans var. *latus* Thom and Raper: Mycologia 31: 657 1939

Colonies on Czapek's solution agar differing from the species in colony development characterized by a felt of predominantly sterile mycelium, few conidial heads, fairly abundant perithecia developed in the mycelial felt and each surrounded by a thick covering of hülle cells (fig. 42 C), very slowly ripening and containing few and scattered asci in abundant sterile mycelium. Ascospore bodies smooth walled, purple red, 3.8 to 4.5 μ by 3.5 to 4 μ with crests 1.5 to 1.8 μ in width.

Type culture NRRL No. 200 received from the Centraalbureau in 1909 and remaining constant in culture since that time. Its antecedent history is not known.

Aspergillus quadrilineatus Thom and Raper Mycologia 31 660, figs 2-4 1939

Colonies on Czapek's solution agar spreading plane or slightly wrinkled with tendency toward floccosity central area gray with a definite purplish tinge, and olive green conidial areas toward the margin occasionally as sectors, perithecia developing separately but abundantly throughout the colony, reverse purplish red, heads short columnar, green, mostly 60 to 70 μ by 30 to 35 μ occasionally larger or smaller, conidiophores sinuate smooth walled dull brownish in color 50 to 75 μ in length by 3.5 to 4.5 μ wide, broadening to 7.5 to 9 μ at the hemispherical vesicular areas primary sterigmata 5 to 6 μ by 3 to 3 μ , secondary sterigmata 5 to 7 μ by 2 to 2.5 μ conidia globose, pale yellow green rugulose, 3 to 4 μ in diameter perithecia enveloped by hülle cells, light brownish in color spherical partially embedded in the mycelial felt (fig 42 D) about 125 to 150 μ in diameter including the enveloping hülle cell layer, with perithecial wall 1-cell layer in thickness, ripening quickly and with ripe asci breaking down to leave the ascospores free ascospores purple red, lenticular with smooth wall, with spore body 4 to 4.8 μ by 3.4 to 3.8 μ , and with two pleated equatorial crests about 0.5 μ in width paralleled by a secondary narrower pair (fig 43 B) which are sometimes indistinct

Type NRRL No 201 (Thom No 4138 N8) from New Jersey soil and kept in culture since 1916 Other strains examined include isolations from Texas Colorado Louisiana, and Maryland

Aspergillus rugulosus Thom and Raper, Mycologia 31 660-663, fig 4 1939

Colonies on Czapek's solution agar slowly and restrictedly growing (fig 41 C) buckled or wrinkled in a mass 2 to 3 mm deep enveloping abundant perithecia at different depths often eventually splitting in the central area purple gray to purple brown in age with green heads sparsely produced and hence not generally evident occasionally seen as small groups and marginal extensions into drying media reverse in shades of deep purple red conidial heads short columnar 75 to 100 μ by 30 to 40 μ conidiophores sinuous smooth walled pale brownish in color 50 to 80 μ long slender, varying up to 5 μ in width, then enlarging to vesicular hemispheres 8 to 10 μ in diameter primary sterigmata 7 to 8 μ by 3 to 3.5 μ secondary sterigmata 5 to 7 μ by 2.5 to 3 μ conidia globose green rugulose 3 to 4 μ

Perithecia very abundant often imbedded in the mycelium as 2 or 3 layers and each surrounded by hyphae and dark brown hülle cells (fig 42 E) globose 225 to 350 μ in diameter including mycelial coverings with dark reddish purple walls of one cell thickness quickly ripening and breaking down to leave ascospores free asci 10 to 11 μ in long axis ascospores purple red lenticular walls conspicuously rugulose (fig 43 C) with spore bodies 4

to 4.4μ by 3.6 to 3.8μ and with 2 pleated equatorial crests with sinuate and entire margins about 0.5 to 0.6μ in width

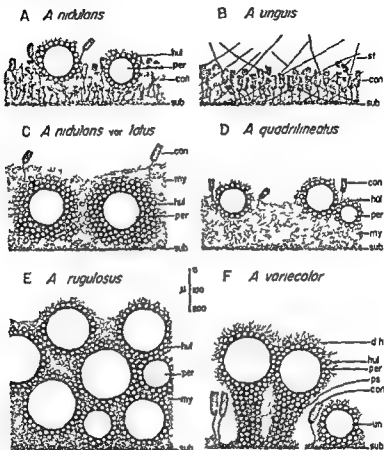


FIG. 42. Diagrammatic representations of cross sections of colonies of different species of the *Aspergillus nidulans* group showing the relative abundance of conidial heads and perithecia and the manner in which these structures are borne: con, conidial heads; dh, mantle of divergent hyphae; hul, hülle cells; my, mycelial felt; per, perithecia; ps, pseudostalk of hülle cells and sterile hyphae; st, long thick walled sterile hyphae; sub, substratum; un, unstalked perithecia. Scale approximate. (Reprinted from Thom and Paper, *The Aspergillus nidulans* Group, Mycologia 51: 653-669, 1939.)

Cultures studied included Type NRRL No. 206 (Thom No. 4138 T11) from New Jersey soil as discussed by Thom and Church in *The Aspergilli*, p. 138 and also isolates from Washington, D. C., Texas, Nebraska and California. Very common in soil.

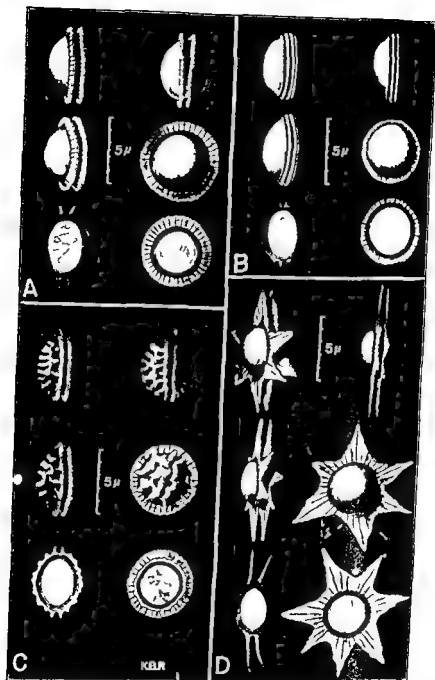


FIG 43 Ascospores of different species of the *Aspergillus nidulans* group. A *A. nidulans* B *A. quadrilinguatus* C *A. rugulosus* D *A. varicolor*. In each species upper left and right and center left spores represent surface profile views center right surface in face view lower left optical section in profile and lower right optical section in face view. (Reprinted from Thom and Raper *The Aspergillus nidulans* Group *Mycologia* 31 653-669 1939)

Culturally and microscopically the above strains present similar pictures with the exception of the strain recently received from Bliss in California (NRRL No 211). In contrast to the others this culture produces abundant conidial heads and relatively fewer perithecia. The ascospores and conidial structures however duplicate those of the typical strains hence we do not at present feel warranted in designating this as a variety, or otherwise separating it from the species *A. rugulosus*.

Aspergillus varicolor (Berk. and Br.) Thom and Raper in *Mycologia* 31: 663-667 fig 4D and fig 5 1939

Synonyms *Emericella varicolor* Berk. and Br. in *Berkely, Introd. Crypt Bot* p 340-341 fig 76 1857. See Patouillard *Bull. Soc. Myc. Fr.* 7: 43-49 pl 4 fig 6-12 1891.
In. engaea erythrospora Borzi *Jahrb. Wiss. Bot. (Pringsheim)* 16: 450-463 pl 19, 20 (1884) 1885.
Emericella medias Choudhury and Mathur *Ann. Myc.* 36: 61-63 1938.
Aspergillus stellatus Curzi *Rend. Acad. Naz. Lincei* 19: 424-428 fig 1 1934.

Colonies on Czapek's solution agar with vegetative mycelium largely submerged sparse spreading slowly in the agar, producing green heads freely in the center of the colony, less abundantly in the outer areas large gray perithecia produced in clusters in colony center and at the margin in some strains, with smaller perithecia scattered through the intervening thinner areas of the colony (fig 44 A) in other strains producing large perithecia abundantly throughout the colony (Pl. IVD) reverse color in shades of purple red. Conidial heads green columnar (fig 44 D) relatively long mostly 100 to 200 μ occasionally up to 300 μ by 30 to 40 μ conidiophores arising directly from submerged hyphae straight with smooth walls cinnamon brown in color mostly 140 to 200 μ long by 3 to 5 μ in diameter broadening gradually to become hemispherical vesicles about 8 to 10 μ in diameter primary sterigmata 7 to 8 μ by 3 to 4 μ secondary sterigmata 8 to 9 μ by 2.5 to 3 μ conidia globose rugulose 3 to 3.5 μ perithecia when clustered (fig 44 As) 300 to 400 μ in diameter surrounded by a felt of hyphae and hülle cells and supported by masses of hyphae and hülle cells forming false stalks (fig 42 F) giving the structures a pyriform appearance (fig 44 B) scattered perithecia much smaller (fig 44 C) and with envelope of supporting cells often much reduced in mass hülle cells abundant and essentially like those of the species *A. nidulans*.

Perithecial wall when stripped of enveloping cells purple red brittle composed of a single layer of cells asci quickly ripening and breaking down to leave the cavity filled with ascospores ascospores purple red with spore bodies lenticular and 3.0 to 4 μ by 2.8 to 3 μ with two prominent equatorial



FIG. 44. *Aspergillus varicolor*. A Central area of colony: a cluster of large pseudostalked perithecia; b scattered smaller unstalked perithecia (see also figure 42 F); c scattered conidial heads $\times 65$. B Enlarged view of large pseudostalked perithecium $\times 65$. C Enlarged view of small unstalked perithecia $\times 65$. D Enlarged view of conidial heads $\times 65$. (Reprinted from Thom and Raper: *The Aspergillus nidulans Group*, Mycologia 31: 653-669, 1939.)

crests, up to 3.5μ in width, pleated and cut to give a stellate appearance to the ascospores (fig. 43 D)

The above description is based primarily upon a culture received from Prof Verona in Italy and carried in our collection as NRRL No 212 (Thom No 56023).

Bliss forwarded a culture (NRRL No 214) isolated from date fruits in California which differs from the above in the following particulars (1) Conical heads are produced abundantly (2) the mycelium is not predominantly submerged and (3) the colonies in reverse are deep purple. However the ascospores of the two strains are strikingly similar in size and pattern the perithecia of each appear pyriform in shape, and the conical structures of the two are essentially alike. More recently a strain has been isolated from Arizona soil (NRRL No 1954) which produces abundant large stalked perithecia but very few small perithecia or conical heads. There is thus evidence of considerable natural variation among members of this species. All of these strains have the same type of stellate ascospore. Since the type of ascospore described here had already been assigned to *Emericella* and *Inengae* consideration of the literature of these genera is necessary.

Emericella varicolor genus and species new was described by Berkeley and Broome in 1857 as doubtfully a *Gasteromyces* or possibly a lichen. The perithecium with a mass of stellate spores was considered as gastro mycetous in character while what we now recognize as the hülle cells of Eidam (figured) suggested to them the possibility of an algal associate. Berkeley a material was also examined by Montagne and part of it deposited in the Museum d'Histoire Naturelle de Paris. This was reexamined by Patouillard in 1891 and its ascomycetous nature determined. No conical apparatus was found by either Berkeley or Patouillard.

Inengae erythrospora as the type species of a new ascomycetous genus was figured and described by Borzi in 1885 showing stellate red ascospores and the hülle cells of Eidam. Borzi's figure showed a coremium like conical apparatus which was designated *Coremium Borzianum* by Saccardo. Ed Fisher in 1893 transferred the species to *Emericella* of Berkeley and placed the genus next to *Aspergillus* in the *Pflanzenfamilien*. Saccardo (in Syll 9 610) on the other hand accepted *Inengae* and dropped *Emericella* because of the errors in description and placement by Berkeley. Borzi figured the spores of *A. varicolor* (*Inengae erythrospora*) correctly but obviously misinterpreted the germination of the ascospores since he showed them splitting as if turned 90° bringing the crest perpendicular to the center of the valve instead of attached to its edges. There the taxonomic situation stood until Willemin in 1927 concluded that the ascospore apparatus the stellate red ascospores and the cells of Eidam as clearly shown in their figures and material showed the identity

of *Emericella* and *A. nidulans*. He therefore transferred *A. nidulans* to *Emericella* as the oldest established genus and apparently did not even consider *In. engae*.

Ciferri (1938) has recently completed a study of *Emericella varicolor* embracing cultural investigations together with a review of the literature of the genus. He did not recognize the close relationship of this fungus to *Aspergillus nidulans* and was apparently unmindful of the likeness of their conidial structures and the essential similarity of their perithecia and ascospores.

In 1934 Curzi described as *Aspergillus stellatus* a fungus characterized by hülle cells and red stellate ascospores. However, he apparently did not know of either Berkeley's or Borzi's earlier designation of a similar fungus. It is unfortunate that his exceedingly descriptive binomial must be reduced to synonymy.

Fortunately for this discussion, cultures NRRL Nos. 212, 214 and 1904 present both the stellate spores and apparently stalked perithecia figured by Berkeley, Patouillard, and Borzi. (Compare figures 42 F and 44, B with Berkeley's figure 76a, Patouillard's figures 7 and 8, plate 4 and Borzi's figure 10, plate 19.) This made possible a restudy of the whole morphologic situation from fresh material. The perithecial body itself was found not to be stalked but to rest upon a sterile mass of hülle cells and mycelium giving the superficial appearance noted by earlier observers.

The coremium of Borzi remains unaccounted for. Obviously in Borzi's discussion the material was rotten olives and very old. No cultures were made. The conidia producing apparatus (figured) differs essentially in type from the conidial apparatus of the *Aspergillaceae* with which Fischer correctly placed *Emericella* because of its perithecia and ascospores. We are convinced that the coremia belonged to some other fungus.

It is not possible to separate the perithecium of this fungus from that of the other species in the *A. nidulans* group nor are there characters to take this type of perithecium out of a genus with the yellow perithecium of the great *A. glaucus* series of species which are widely known. Consistent with the policy of keeping the *Aspergilli* in one group both *Emericella* and *In. zengae* are dropped for purposes of this discussion.

Aspergillus caespitosus Raper and Thom in *Mycologia* 36: 563-565
fig. 4 1944

Colonies varying markedly upon different media, upon Czapek's solution agar rather slow growing attaining a diameter of 6 to 8 cm. in three weeks at room temperature. plane or somewhat furrowed mycelium largely submerged and extremely tough tearing with difficulty producing numerous dark green hemispherical to loosely columnar heads in central colony areas.

characterized particularly by clusters of irregular ovoid to elliptical thick walled hülle cells at first colorless becoming reddish purple in age scattered

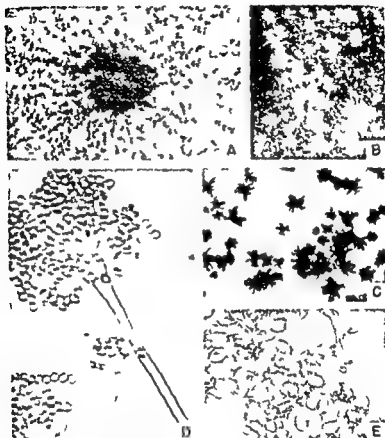


FIG. 45 *Aspergillus caespitosus* NRRL No. 1979. A Portion of colony on Czapek's solution with 1 percent liver extract, showing crowded conidial heads in central portion and scattered hülle cell masses in surrounding areas two weeks old incubation at room temperature $\times 1.8$. B Portion of colony enlarged showing hülle cell masses $\times 10$. C Silhouettes of conidial heads developed on hay infusion agar $\times 111$. D Typical conidial head showing form of vesicle and arrangement of sterigmata $\times 600$. E Portion of hülle cell mass showing irregular size and form of component elements $\times 265$. (Reprinted from Raper and Thom, New Species of *Aspergillus* from Soil, Mycologia 36 Nov.-Dec. 1944.)

unevenly (fig. 45 A and B) or arranged in irregular concentric zones reverse colorless at first becoming dark reddish purple in age particularly beneath the hülle masses odor none Conidial heads dark dull yellow green to

empire green (Ridgway, Pl XXXII) generally hemispherical to loosely columnar, mostly 75 to 125 μ in diameter. Conidiophores straight or slightly sinuous (fig 45 D), mostly 250 to 325 μ in length, occasionally up to 350 μ by 50 to 65 μ in diameter of approximately uniform diameter throughout relatively thick walled (12 to 15 μ in basal portion to 08 to 10 μ in terminal area), smooth, tan to light brown in color. Vesicle slightly elongate, the upper hemisphere loosely covered by sterigmata (fig 45 D) the lower half sterile and often lightly colored, mostly 15 to 20 μ in diameter. Sterigmata in two series (fig 45 D) primaries normally 6.5 to 8.5 μ by 3.5 to 5.0 μ , secondaries 6.5 to 8.0 μ by 3.0 to 4.5 μ typically bottle form but commonly much swollen and often quite irregular in form and dimensions. Conidia globose, spinulose, green, mostly 3.5 to 4.5 μ rarely larger, hülle cells very abundant thick walled, irregularly globose ovoid or elliptical (fig 45 E) ranging from 12 to 18 μ in globose cells to 12 to 15 μ by 25 to 30 μ in the most elongate bodies forming compacted masses of indefinite size extremely tough and in age becoming almost sclerotoid at first colorless but in age characterized by an abundant reddish purple intercellular pigmentation.

Colonies upon malt agar characterized by a dense stand of erect conidiophores bearing hemispherical to radiate or loosely columnar heads of dark green color approximately empire green (Ridgway, Pl XXXII) and the complete absence of hülle cells, reverse in light brown shades odor none. Details of morphology as upon Czapek's solution agar.

Colonies upon hay infusion agar like those upon malt except less heavily sporing.

Strains include NRRL No 1929 (type) isolated from Arkansas soil and other isolations from Arizona and Texas soils.

This species is of particular interest because of its apparent transitional position between the *A. nidulans* group and *A. ustus*. In the character of its conidiophores its reddish purple pigmentation, and in the general color and markings of its conidia it retains the characters of *A. nidulans* and closely related species. In the absence of fertile perithecia and ascospores the predominantly hemispherical shape of its conidial heads, and in the variable and irregular form of its hülle cells it is strongly suggestive of the *A. ustus* series. While we are convinced of its intermediate position between the *A. nidulans* group and *A. ustus* we place it with the former since we believe it is most closely allied to this group. It is believed significant that superficially cultures of *Aspergillus caespitosus* and *Aspergillus variegator* (Berk. and Br.) Thom and Raper (1939) are strikingly similar upon Czapek's solution agar. This similarity is particularly marked when plates are viewed in reverse since an intense pigmentation marks the under surface of perithecia in the latter case and the under surface of older hülle masses in the former.

Aspergillus unguis (Emile Weil and Gaudin) Emend Thom and Raper,
Myc 31 p 667 fig 6 1939

Synonym *Sterigmatocystis unguis* Emile Weil and Gaudin Arch Med
Expt Anl Path Paris 28 463-465 fig 4 1919

A. loakianensis Shih Lingnan Sci Jour II (3) 369 p
16 fig 2 1936

Colonies on Czapek's solution agar restrictedly growing plane spreading at the margin as irregular lobes (fig 41 E) yellowish green green to dark green becoming brown in age, without perithecia or hülle cells Mycelial preparations show striking sterile thick walled hyphae with walls in brown



FIG 46 Sterile spicule hyphae of *Aspergillus unguis* A, Cluster of sterile hyphae $\times 30$ B Apex of sterile hypha $\times 740$ C and D Mid portions of sterile hyphae showing thick roughened walls $\times 740$ (Reprinted from Thom and Raper The *Aspergillus nidulans* Group Mycologia 31 653-663 1939)

shades irregularly roughened (fig 46) tapering to a blunt point arising sometimes from foot-cells suggesting the origin of conidiophores sometimes apparently from mycelial cells often up to 1000μ or more in length slanting upward but usually rising only slightly above the conidial area (fig 42 B)

Conidial heads columnar 75 to 150μ by 40 to 50μ conidiophores smooth walled dull brown in color mostly 45 to 65μ in length by 3 to 5μ in diameter enlarging to vesicular hemispheres 9 to 12μ in diameter primary sterigmata 5 to 6μ by 2.5 to 3μ secondary sterigmata 5 to 6μ by 2 to 2.5μ conidia globose rugulose, dull green 2.5 to 3μ in diameter

Cultures of *A. unguis* are obtained frequently from medical laboratories apparently as more or less active pathogens but occasionally isolated from

soil and decaying organic matter. The question whether the non ascospore members of the group have merely dropped the ascogenous phase or constitute a separate species was answered when more complete examination showed the sterile or spicule hyphae to be regularly produced in the non ascospore but never found in ascospore series.

Pathogenicity

Aspergillus nidulans in some of its forms and variants has been demonstrated as a parasite in human nails (onychomycosis), often enough to establish its pathogenicity. *A. nantae* Pinoy (1927) probably belongs here although the data are mainly pathological, hence not adequate for definite identification of the organism. *A. nidulans* forme *cesarii* Pinoy (1915) isolated from a mycetoma of the lung of a donkey, and *A. nidulans* var. *nicolletii* Pinoy (1906) isolated in Tunis from a case of mycetoma, or madura foot, represent additional strains which were at least secondary pathogens. The nearly related *A. unguis* is usually the more common form isolated from human material. *A. Brodeni* (Mattlet) Dodge (1930) from a bronchomycosis in Africa might have been close to *A. unguis*. *A. nidulans* and *A. unguis* are both widely distributed as saprophytes hence are constantly encountered as components of dirt reaching the extremities by contamination. Infection of the air passages is comparatively rare.

Occurrence and Economic Importance

In addition to their role as occasional disease producing agents members of the *A. nidulans* group are believed to be significant in decomposition processes. They are among the molds most commonly isolated from soil, and very frequently appear in considerable abundance upon vegetable material undergoing slow decomposition. *Aspergillus rugulosus*, *A. quadrilineatus* and *A. caespitosus* occur most frequently in soils from the comparatively dry warm soil of Texas, Arizona and adjoining areas. *Aspergillus varicolor* has been isolated from olives in Italy, date fruit in California, and from Arizona soil. *Aspergillus nidulans* is abundant and cosmopolitan in its distribution.

CHAPTER VII

THE ASPERGILLUS USTUS GROUP

Outstanding Characters

Colonies more or less floccose at first white but becoming dull in age in most members varying from olive gray through reddish brown to fuscous as conidial structures develop

Conidiophores in yellow brown shades smooth

Heads irregular in form ranging from more or less radiate to hemispherical to loosely columnar

Vesicles hemispherical sterigmata in two series loosely arranged

Conidia roughened 30 to 50 μ varying from echinulate to marked with conspicuous color bars and ranging in color from pale blue green through olive green shades to deep brown (fuligineus)

Hülle cells regularly present thick walled elongate often more or less curved and twisted

Included here are representatives of a most abundant and widespread group of fungi especially common in soil and upon decaying vegetation

Group Key

Colonies predominantly floccose heavy sporing hülle cells not aggregated in small clusters *A. ustus* (Bain.) Thom and Church

Colonies more or less floccose light sporing hülle cells aggregated in small clusters *A. granulatus* Raper and Thom

Aspergillus ustus (Bainier) Thom and Church in The

Aspergilli p 152 1926

Synonym *Sterigmatocystis usta* Bainier in Bul Soc Bot France 78

1881

Colonies upon Czapek's solution agar spreading broadly plane sulcate or umbonate rarely zonate more or less felted or floccose, at first white becoming olive gray, yellow brown fuscous or russet to purplish vinaceous with the development of mature conidial structures (Pl IV E, and figs 48 A and B) generally heavy sporing with some conidiophores arising from the substratum but more abundantly from aerial hyphae reverse in shades of yellow orange, and brown to almost black in age odor not pronounced Heads radiate to irregularly hemispherical sometimes loosely columnar commonly splitting into more or less well-defined columns in age very variable in size ranging in color from dull green or olive gray through gray

ish brown to fuscous or fuliginous. Conidiophores arising from submerged hyphae (fig 47 A) ranging up to 500μ long by 3 to 6μ aerially borne conidiophores, ranging from very short (fig 47 A₁) up to 125μ by 2 to 5μ sinuous sparsely septate, with walls rather thin, smooth, and uniformly colored some shade of brown. Vesicles hemispherical to subglobose 8 to 20μ in diameter, smaller in some strains (fig 47 A). Sterigmata colorless or

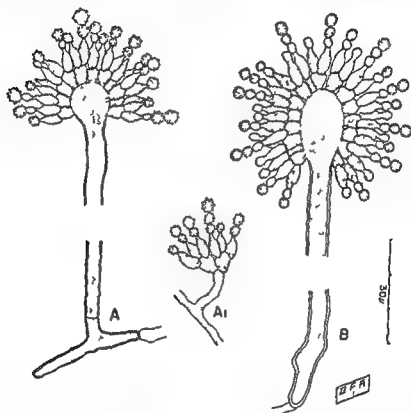


FIG 47 *Aspergillus ustus* group $\times 840$. A Typical conidial head showing comparatively loose sterigmata in two series and conspicuously roughened conidia strain NRRL No 278. A₁ Diminutive head as often seen in strain NRRL No 278. B Typical head of *Aspergillus granulatus* NRRL No 193.

colored semi radiate loosely arranged into two series primary sterigmata 4 to 7μ by 3μ secondary sterigmata 8 to 7μ by 2.0 to 2.5 μ . Conidia globose 3.5 to 5.0 μ roughened echinulate to marked with conspicuous color bars ranging from greenish through olive gray to yellow brown or fuliginous. Many strains producing thick walled hülle cells (fig 49 E) ranging in form from irregularly ovate or elongate in some strains to serpentine helicoid or twisted in others essentially as in *Aspergillus flammipes*.

Upon malt agar, colonies frequently heavier sporing and conidial heads generally tending to run to dull gray green shades rather than brown

Very common in soil and decaying vegetation Species diagnosis represents a composite based upon many isolations from this country and abroad

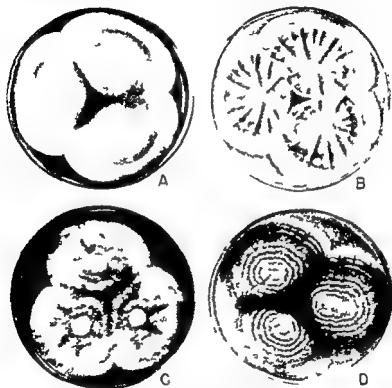


FIG 19 *Aspergillus ustus* cultures growing upon Czapek's solution agar at room temperature 10 days A Strain NRRL No 275 characterized by loose floccose colonies and moderate sporulation B Strain NRRL No 278 characterized by heavier spore production and the presence of abundant hülle cells C *A. ustus* var *laevis* NRRL No 1859 characterized by loose floccose colonies and conidial heads often near brick red in color D Strain NRRL No 1974 characterized by the production of very abundant hülle cells in concentric zones

Individual strains differ markedly in their general habit and colony coloration in the color of their fruiting structures in the marking and coloration of their conidia in the presence or absence of hülle cells and in the form of these structures when present By a deliberate selection of strains one can find sufficient difference to warrant the assignment of specific designa

tions to particular cultures. Yet all possess the characters noted above and so constitute a well-defined group whose variations are matters of detail. Such differences as occur, moreover, tend to become bridged as comparative cultural and microscopic studies of many isolations are made, hence the use of these differences becomes of questionable value for diagnostic purpose. We have considered it desirable, therefore, to include the whole series under one name as a single species aggregate *Aspergillus ustus* and to call attention to some of the major differences which one may expect to encounter among the members of this species. Although Bainier was not sufficiently explicit in his description of *Sterigmatocystis usta* to enable us to identify with certainty the form with which he worked, his usage is accepted upon (1) the basis of priority, and (2) the receipt of a culture (Thom No 4640 488) from his laboratory labeled *Sterigmatocystis usta* which possessed the basic characters of the group as herewith set forth.

Long after the publication of *Sterigmatocystis usta*, Bainier described a second species belonging to this group, *Sterigmatocystis insueta* (Bul Soc Mycol France 24 85-87, Pl VIII Fig 1-13 1903) and emphasized the fuligineous character of its colonies, the predominant origin of brown fruiting structures from aerial mycelium, the larger size and the darker color of its conidia which were characterized by the presence of pronounced color bars. Strains showing these characters probably represent the type most commonly encountered among miscellaneous isolations of forms belonging to this group. Recognition of these forms as constituting a distinct species has been considered, but in the absence of any clearly definable line of separation from *A. ustus* it is believed desirable to leave them within the somewhat extended framework of this species. Cultures possessing these characteristics very commonly exhibit hülle cells varying in different strains from ovoid to irregularly elongate to serpentine, helicoïd or otherwise twisted. One strain showing much twisted hülle cells was isolated and contributed by Thaxter under the manuscript name *A. helicophorus*. Typically the conidiophores of these forms are grayish brown, commonly quite dark. The vesicle and sterigmata are likewise frequently colored. Conidial color as seen under high magnifications varies with age from pale to olive green to fuligineous, and conidial markings from fairly coarse echinulations to intensively colored bars and tubercles.

Possibly unaware of the existence of *Sterigmatocystis usta* and *S. insueta* (since no mention is made of either species) Abbott (1926) subsequently described *Aspergillus minutus*. His description indicated that he was dealing with a strain essentially similar to those considered by Bainier as *S. insueta*. This is confirmed by examination of his type culture NRRL No 283 (Thom No 4894 2). A second culture NRRL No 285 (Thom No 4894 1) received from Abbott under the manuscript name *A. humus* likewise represents a member of this series and differs from the more

common forms only in its more floccose habits and in the production of somewhat smaller conidial heads

Among the more striking members of the *Aspergillus ustus* series examined are two isolations from soil collected in Panama and Mexico respectively, which upon Czapek's solution agar normally produce strongly zonate colonies consisting of alternating areas of crowded conidial heads and heavy hülle cell development (fig 48 D) Except for this difference in colony appearance, however these strains appear to be typical of the species as described above

A single strain NRRL No 1852 isolated from Louisiana soil possesses conidial heads of a dull brick red color (Ridgway Pl XXXIX russet vinaceous to sorghum brown) and abundant much twisted hülle cells While Blochwitz's description is too inadequate to permit of detailed comparison it is suggested that this strain may represent his *Aspergillus ustus* var *laevis* (Ann Mycol 32(1/2) 4 1934) which was described as characterized by red conidia and crooked hülle cells In strain NRRL No 1852 the conidia are conspicuously reddish en masse but appear only slightly colored when viewed with high magnifications In contrast to most strains of the *Aspergillus ustus* group the conidia are finely echinulate rather than coarsely roughened It is suggested that this form (fig 48 C) may represent a transition in the direction of *Aspergillus flavipes* since the latter species is likewise characterized by much twisted hülle cells brown conidiophore walls and conidia which may show a reddish color in some strains but are smooth in all

Aspergillus granulosus Raper and Thom in Mycologia 36 565-568
fig 4 1944

Colonies upon Czapek's solution agar growing well attaining a diameter of 8 to 10 cm in two to three weeks at room temperature plane or irregularly furrowed predominantly floccose uneven in texture buff to dull brown in color from felted sterile mycelia conidial heads few in number and generally arising from the substratum direct less often from aerial hyphae commonly appearing in clusters pale blue green in color colonies characterized particularly by abundant small colorless clusters of irregularly globose ovoid or elliptical thick walled hülle cells which superficially suggest perithecial initials and which in mass give to the colony a semi granular appearance (fig 50 B E and F) reverse in shades of dull yellow and brown slight mushroom odor Conidial heads few in number commonly clustered in small groups most abundant at colony margin sometimes occurring on tufts of aerial hyphae hemispherical to radiate 75 to 125 μ in diameter very loose consisting of comparatively few divergent spore chains (fig 50 C) approximately pale niagara green in color (Ridgway Pl XXXIII) Conidiophores erect straight nonseptate mostly 350 to

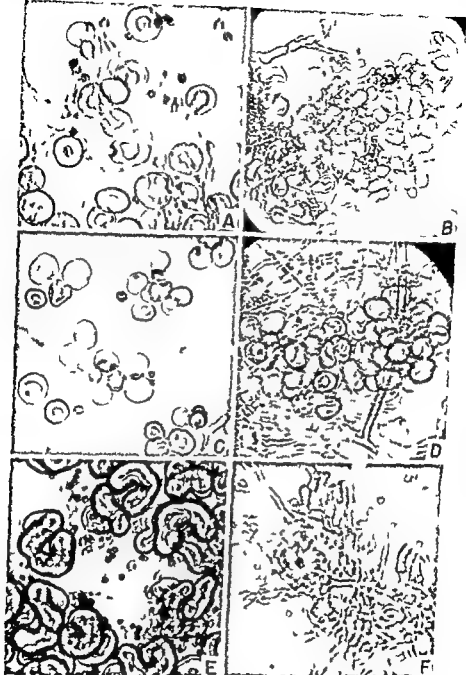


FIG. 49. Hülle cells. A Characteristic thick walled globose to subglobose hülle cells of the *Aspergillus nidulans* group A *varicolor* NRRL No 1901, $\times 150$. B *Aspergillus caespitosus* strain NRRL No 1930 hülle cells irregular in form and often poorly developed but of the same general pattern as *A. nidulans* $\times 25$. C *Aspergillus janus* NRRL No 187 hülle cells approximately globose and of the same basic type as in *A. nidulans* $\times 450$. D *Aspergillus granulosis* NRRL No 1932 hülle cells globose to ovoid somewhat intermediate between *A. nidulans* and *A. ustus* $\times 275$. E *Aspergillus ustus* NRRL No 280 characteristic hülle cells elongate and much twisted $\times 450$. Hülle cells in *Aspergillus fijiensis* are of the same pattern. F *Aspergillus caesus* NRRL No 1976 heavy walled strongly septate mycelium suggestive of hülle cells $\times 775$.

500 μ in length 55 to 80 μ in diameter approximately uniform in width throughout thin walled smooth tan to light brown in color often slightly

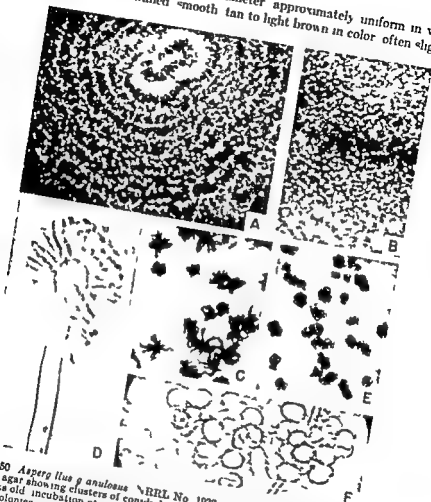


FIG 50 *Aspergillus granulatus* NRRL No 1937 A Portion of colony on hay infusion agar showing clusters of conidial structures and small masses of hülle cells of two colonies on malt extract agar showing characteristic granular appearance resulting from numerous small clusters of hülle cells B Portion of marginal area of conidial heads C Silhouettes of conidial heads D Conidial head showing slightly elongate vesicle sterigmata in two series and constriction in conidiophore just beneath the vesicle E Small clusters or granules of hülle cells F Double cluster of hülle cells much enlarged

X 265 (Reprinted from Raper and Thom New Species of Aspergillus from Soil Mycologia 36 Nov-Dec 1944)

constricted just beneath the vesicle (figs 47 B and 50 D) Vesicle ovoid to elliptical thin walled and easily broken largely covered by sterigmata 12 to 18 μ in diameter by 15 to 25 μ in length Sterigmata in two series

both comparatively short and stout primaries 3.5 to 5.0 μ by 3.0 to 4.0 μ , secondaries 4.0 to 5.5 μ by 3.0 to 3.5 μ commonly bottle form. Conidia globose, pale green delicately echinulate. Mostly 4.8 to 5.5 μ in diameter, rarely larger. Hulle cells abundant, irregularly globose ovoid or somewhat elongate, commonly 12 to 30 μ in long axis walls heavy, 4 to 5 μ in thickness borne primarily in small colorless clusters which are quite conspicuous at colony margins and lend to them a characteristic granular appearance.

Colonies upon malt extract agar showing an accentuation of hulle cell development (fig. 50 B) and a reduction in conidial heads. Otherwise duplicating the cultural picture presented upon Czapek's solution agar.

Colonies upon hay infusion agar (fig. 50 A) thin but broadly spreading characterized by scattered clusters of hulle cells and erect conidial fructifications giving to the culture a sparsely granular appearance.

Type culture NRRL No. 1932 was isolated in 1942 from a sample of soil collected in Fayetteville, Arkansas, and contributed by Mr. F. R. Earle. Additional strains have been isolated from soils collected in Texas, Arizona and Costa Rica. It is believed common in soils where the temperature remains at a high level during part or all of the year.

Different strains vary materially in the number of conidial heads produced upon common laboratory media such as Czapek's solution and malt extract agars ranging from abundant heads in some to only widely scattered heads in others. All fruit reasonably well however upon hay infusion agar, the medium upon which original isolations were made.

The brown color of the conidiophores, the presence of ovoid to somewhat irregular hulle cells, and the green color of its conidia place this species in the group with *A. ustus*. It differs markedly from the more common representatives of this group however, in the lighter and persistently green color of its conidia, the small clusters rather than irregular masses of hulle cells and in possessing somewhat more elongate vesicles. In this latter character it suggests *Aspergillus flavipes* but is in turn excluded from this group by the green color of its spores.

Occurrence and Economic Importance

Representatives of the *Aspergillus ustus* group are perhaps the most abundant of all aspergilli in soil. They regularly occur in large numbers and in considerable variety. The common species *A. ustus*, occurs alike in cultivated and forest soils and in approximately equal abundance in soils from southern and from north temperate areas. *A. granulatus* on the contrary, has been isolated only from southern sources. Members of the group are not known to be particularly active agents of decomposition but their great abundance in nature is believed indicative of a significant role in many decay processes.

Their biochemical activities and potentialities are almost completely unknown.

CHAPTER XIII THE ASPERGILLUS FLAVIPES GROUP

Outstanding Characters

- Conidiophores smooth in some shade of yellow with color often confined to the outer layer
- Heads barrel form to columnar when well developed white or slowly becoming some shade of vinaceous buff to avellaneous (Ridgway Pl XL)
- Vesicles subglobose to elliptical
- Conidia colorless smooth thin walled
- Hülle cells generally present helicoid or variously twisted

The range of variation presented within the group has led workers with only a few representatives before them to offer names and descriptions for the strains under observation. However when large numbers of strains are brought together and cultivated upon a considerable range of substrata the continuity of the group as a natural and related series of strains becomes apparent. Further study of this whole group may lead to separation upon lines accounting for certain published descriptions not at present identifiable. For purposes of the present manual however it is believed desirable to broaden the description of *Aspergillus flavipes* (Bain and Sart) Thom and Church sufficiently to include a closely related series of organisms rather than restrict it to the particular strains studied by Bainier.

Aspergillus flavipes (Bain and Sart) Thom and Church
The *Aspergilli* p 155 1926
90-96 Pl III fig 1-6 1911)

Synonym *S. flavipes* Bainier and Sartory (Bul Soc Myc France 27
Colonies upon Czapek's solution agar rather slow growing becoming 3

to 5 cm in diameter in about 10 days mycelium yellowish dull buff commonly becoming brownish in age heads pale to dull buff in some strains to avellaneous or even very light cinnamon in others (Pl I) F and fig 81 A) submerged mycelium persistently colorless in some strains developing many shades of yellow orange orange brown to red (Madder brown of Ridgway Pl XIII) or almost black in reverse of colonies in others in some strains producing at the surface of the agar closely woven yellow to orange masses of hyphae encircling numerous helicoid or variously twisted thick walled hülle cells occasional strains showing dark masses

suggestive of *sclerotia* aerial mycelium more or less abundant colorless or yellow to orange, producing in many strains numerous large drops of transpired fluid, pale to yellow or orange red (acting as an indicator, changing from yellow with acid to orange red with alkali) commonly

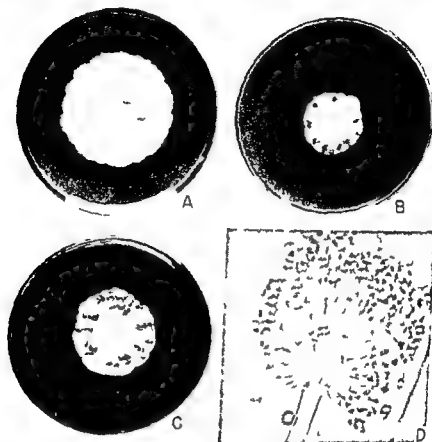


FIG 51 *Aspergillus flavipes* group A *A. flavipes* NRRL No. 295 growing on Czapek's solution agar 2 weeks room temperature B Strain NRRL No. 237 of the same species found by White (1943) to produce a penicillin like substance C, Strain NRRL No. 1959 characterized by the production of an excessive amount of exudate D Photomicrograph of a typical head showing the elongate vesicle and crowded sterigmata in two series $\times 750$

characterized by a disagreeable odor approaching putridity Heads typically becoming columnar masses shading from persistently white, through shades of pale to deep avellaneous as in *A. terreus* but usually in rather sharp contrast to the color of the mycelium Conidiophores from 300 to 500 μ by 4 to 5 μ in crowded areas up to 2 to 3 mm in length and

8 to 10 μ in diameter in some strains and under some conditions of culture walls more or less yellow under the microscope with color mostly localized in the outer layers of the cell wall and occasionally as disk like concretions on the surface of walls otherwise smooth. Vesicles subglobose to elliptical (fig 51 D) up to 30 by 40 μ in the largest forms usually with diameter twice that of the conidiophore in smaller forms. Sterigmata in two series colorless or nearly so closely packed over the apex of the vesicle in small heads and covering the vesicle in large heads primary sterigmata about 6 or 8 μ by 2 to 3 μ secondary sterigmata 5 to 8 μ by 1.5 to 2 μ . Conidia 2 to 3 μ smooth subglobose colorless or nearly so under high magnification with chains aggregated to form columns as seen with the handlens in old cultures.

Cosmopolitan in distribution and particularly common in soil and upon decomposing organic materials.

Historically the name applied to the series is taken from strains 4640 474 and 4640 402 (Thom Collection) obtained through daFonseca from the Bainier collection in Paris in 1922. These strains showed smooth yellow conidiophores 300 to 400 μ by 3 to 4 μ contrasting with heads that were rather persistently white and possessed the general morphology of the series as described above. Hülle cells were not found. Nevertheless the close relationship of these organisms to a great series of cultures obtained from many sources in which these structures are regularly found justifies us in broadening the use of the name.

Culture No 4640 486 (Thom) received from the Bainier collection as *S. rubescens* (NRR, No 291) shows deep floccose colonies with few heads and scattered dark hyphal masses in age. Hülle cells have not been seen in this strain.

Among forms commonly obtained from soils collected from widely scattered areas in this country and abroad a series of isolates seems to comply with the description given by Blochwitz for *Aspergillus archi flavipes* (Ann. Mycol. 32(1/2) 84 1934). This species as described represents an extreme development toward radiate heads abundant conidia conidiophores 2 to 3 mm in length and the development of deep brown or actual red shades of color in the mycelium. Several strains observed in culture approach this description (fig 51 C). Heads are at first globose then become slowly barrel form i.e. short stocky columns. Large brown drops of transpired fluid are commonly seen which become yellow when acidified and return to reddish shades when alkali is added. Recognition of the species does not appear warranted since no clearly definable character exists which distinguishes these isolates from the less colored forms generally considered as representing *Aspergillus flavipes* in a more restricted sense.

Antibiosis

Working with a culture of *Aspergillus flavipes* from Thom (No 4303 46), NRRL No 287, White (1943) has recently demonstrated the production of an antibacterial substance which in its action against *Staphylococcus aureus* strongly resembles penicillin. The substance was produced in greatest amount in a medium containing 5 to 10 percent corn steeping liquor as the sole nutrient. The addition of sugar is reported as definitely deleterious.

Occurrence and Economic Importance

Members of the *Aspergillus flavipes* group like those of the *A. ustus*, *versicolor*, and *terreus* groups are cosmopolitan in distribution and are especially common in fertile soil and upon decaying vegetation. They are not known to be active agents of decomposition but are capable of growing in the presence of a limited amount of water, hence are probably significant in initiating or continuing processes of decay where most micro-organisms are incapable of growing. Little is known of the biochemical activities or products of these forms.

CHAPTER XIV

THE ASPERGILLUS VERSICOLOR GROUP

Outstanding Characters

- Conidial heads hemispherical to almost globose in many different shades but usually showing green or blue green
- Conidiophores smooth, colorless, more or less sinuous
- Vesicles globose to ovate or elliptical with radiate sterigmata borne over the upper half to three fourths of the surface
- Sterigmata in two series
- Spores globose or subglobose echinulate
- Hülle cells found in occasional strains globose

Members of the *Aspergillus versicolor* group are cosmopolitan in distribution. They occur regularly in soil, upon decaying vegetation upon stored grains upon cured meats and upon a multitude of other products exposed to occasional moist air or undergoing slow decomposition. The morphology of all strains (with the exception of *Aspergillus janus*) is basically alike but different strains vary tremendously in their cultural appearance. This is especially true of *Aspergillus versicolor* where conidial heads in different strains vary in color from dark blue-green through green to yellow-green to yellow and orange and finally in some strains to yellowish-cream buff or flesh color.

Group Key

- I Heads typically globose less commonly hemispherical blue green in color with the blue element dominant colony reverse and substratum usually in red or maroon shades *A. sydowii* series
 - A Conidial heads always blue green globose to radiate *A. sydowii* (Bain and Sart.) Thom and Church
 - B Conidial heads of two types (a) blue green in color vesicles subglobose to elongate borne on short conidiophores and (b) white clavate borne upon long conidiophores *A. janus* Raper and Thom
- II Heads hemispherical or nearly globose at times in green shades without blue admixture buff to orange yellow or occasionally flesh colored colony reverse usually in pink yellow red or purple red shades rarely almost colorless *A. versicolor* series
 - A Conidia echinulate *A. versicolor* (Vuill.) Tiraboschi
 - B Conidia smooth *A. fumicola* Chad. and Sach.

Aspergillus sydowii (Bain and Sart) Thom and Church The Aspergilli, p 147 1926

Synonym *S. sydowii* Bainier and Sartory, in Ann Mycol 11 25-29, Pl III 1913 Compare Pl IV cultures No 4235 17 and A22, Thom and Church The Aspergilli 1926

Colonies upon Czapek's solution agar growing well at room temperature, in most strains close textured and velvety from crowded conidiophores and heads arising from the substratum (fig 53 A), in other strains more or less



FIG 52 Conidial structures in *Aspergillus sydowii* NRRL No 250, $\times 800$ A Typical large conidial head borne upon an erect conidiophore arising from the substratum B Small head borne as a lateral branch on an aerial hypha consisting of a small cluster of double sterigmata C Minute heads in which sterigmata appear in a single series only

floccose (fig 53 B) from interlacing and trailing aerial hyphae bearing conidial heads ranging from large, well formed structures to minute fruits consisting of clusters of simple sterigmata bearing few conidial chains blue green in color approximately Delft blue or deep Delft blue (Ridgway Pl XLII) with the blue effect especially marked in young fruiting areas (Pl V A) reverse usually in shades of red from coral red to maroon (Ridgway

Pls VIII and I) to almost black in some strains in age. Conidial heads typically radiate to nearly globose (fig 52 A) ranging from 100 to 150 μ but often reduced to small penicillate clusters of sterigmata especially in marginal colony areas and upon aerial hyphae (fig 52 II and C). Conidio-

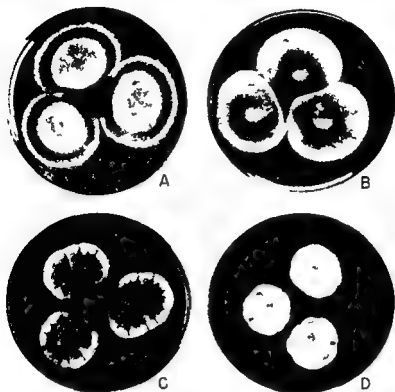


FIG 53 Colony types in the *Aspergillus versicolor* group on Czapek's solution agar, 10 days room temperature. A Typical heavy sporing strain of *A. sydowii*. B Floccose lighter sporing strain of the same species. C *A. versicolor* NRRL No 239 characterized by abundant green conidial heads and the production of deep red exudate. D *A. versicolor* NRRL No 277 characterized by flesh-colored conidial heads.

phores mostly arising from submerged hyphae up to 500 μ in length by 5 to 8 μ in diameter colorless, smooth comparatively thick walled. Vesicles nearly globose fertile over almost the entire surface up to 20 μ in diameter. Sterigmata in two series primary 6 to 7 μ by 2 to 3 μ secondary 7 to 10 μ by 2 to 2.5 μ . Conidia globose 5 to 3.0 μ (Barnier) in our culture up to 3.5 μ in diameter conspicuously spinulose green en masse. Globose hülle cells

closely resembling those of the *A. nidulans* group have been seen in occasional strains. No sclerotia or perithecia are found, but clamydospores in solid substrata are reported.

A typical member of our series of isolations was sent to Baimier who concurred in our interpretation of his species.

Reduced conidial apparatus appears in varying degree in all strains of *A. sydowii* examined. In typical strains primary sterigmata with their clusters of secondaries each bearing a chain of conidia are found singly or variously grouped along trailing aerial hyphae. In other strains there is a progressive development of aerial mycelium in the form of trailing hyphae either single or in ropes, coupled with a reduction in the number of typical *A. sydowii* heads. Strains are even occasionally seen in which only a few *A. sydowii* conidiophores and heads are found in what is otherwise a pencilium like colony. Thus a series of strains exists which shows a fairly complete gradation from Baimier and Sartory's *Sterigmatocystis sydowii* to *Penicillium restrictum* of Gilman and Abbott. We are led to believe that the latter species should probably be assigned to this section of the genus *Aspergillus*.

While morphologically very close to *A. versicolor*, *A. sydowii* is easily presumptively recognized by the characteristic blue green color of its conidial heads and the red colors in the substratum. A partial list of the sources of the isolations studied includes soil in Washington D. C., Illinois, Manitoba, Florida and Ceylon, moldy silk from a stocking factory, concentrated sugar products from Louisiana, dried fish in Japan and bee hives in Michigan. It is world wide in distribution and very adaptable to substrata of widely different nature.

Probable Synonyms

Aspergillus tiroboschii Carbone (Atti d. Inst. Bol. Univ. Pavia Ser. II Vol. XVI p. 320 1914) is described in the colors of *A. versicolor* but with the head of *A. sydowii*. It would appear to be more or less intermediate but unless reisolated must be dropped because of incomplete data.

Sterigmatocystis tuncelana Langeron (Bull. Soc. Path. Exot. 17 315-317 text fig. 1924). This mold was recorded as isolated from an ulcer of the hand but failed to produce lesions in animal tests as described the colonies were blue green as in *A. sydowii*.

A. sydowii var. *achlamydosporus* Nakazawa, Sino and Watanabe (Jour. Agr. Chem. Soc. Japan 10(2) 178-179 1934). The absence of chlamydospores (hülle cells?) in a strain of *A. sydowii* is hardly a sound basis for separation.

Sterigmatocystis cyaneus Mattliet (Ann. Soc. Belg. Med. Trop. 6 32 1926) was described without data to separate it from *A. sydowii*.

S. cameleo Sartory Sartory and Meyer (Ann. Mycol. 29 360-361 Pl. III figs. 7 & 8 1930) by description must have been some strain of *A. sydowii* although the very small smooth conidia do not agree. It may possibly represent a form near *A. humicola* as described by Chaudhuri and Sachar (see p. 193).

Aspergillus janus Raper and Thom in *Mycologia* 36: 556-561, fig. 1. 1944

Species characterized by conidial heads of two distinct types (1) large white heads borne upon long conidiophores terminating in strongly clavate vesicles and (2) smaller dark green heads borne upon short conidiophores with typically ovate vesicles (Pl. I E and F, and V B)

Colonies varying greatly in color and in texture depending upon the substratum and the temperature of incubation. Upon Czapek's solution agar at 24° C (fig. 54 A) colonies spreading irregularly usually consisting of a central floccose mass 1 to 3 mm deep pale yellow buff in color bearing few and scattered fruiting structures surrounded by an irregular zone of crowded fructifications with dark green heads occurring in a dense stand adjacent to the substratum (fig. 54 D) and with numerous long stalked white heads projecting above this layer (fig. 54 C) reverse in dull yellow to light brown shades. When incubated at 20° C colonies more restricted, less floccose and consisting almost exclusively of a dense stand of long stalked white heads with small green heads absent or developing only in age and arising from trailing asexual hyphae entwined among the white fruiting structures. When incubated at 30 to 32° C colonies close textured predominantly green but with central area commonly showing irregular patches of massed hülle cells buff to dull yellow in color. Conidial heads abundant and consistently dark green in color. Reverse in dull brown shades.

White conidial heads loose in texture (fig. 54 C) consisting of radiating and divergent chains of conidia commonly 150 to 200 μ in diameter occasionally larger. Conidiophores long thin mostly 2 to 2.5 mm in length by 8 to 10.5 μ in diameter occasionally larger, erect essentially uniform in diameter throughout but often marked by numerous and irregularly spaced constrictions walls smooth colorless approximately 1 to 1.4 μ in thickness. Vesicles thin walled clavate (fig. 54 G) mostly 45 to 60 μ by 15 to 18 μ with individual structures larger or smaller entire surface loosely covered by sterigmata as a rule but often showing barren areas which may occupy any part of the sterigmatic surface. Sterigmata in two series primaries 7 to 10 μ by 3.5 to 4.5 μ secondaries 6 to 8 μ by 2.5 to 3 μ . Conidia smooth, colorless globose to subglobose mostly 2 to 2.5 μ with maximum about 2.8 μ .

Green conidial heads compact radiate when young becoming columnar in age and often spreading into two divergent columns. Heads at first in blue to blue green shades near dark goblin blue (Ridgway Pl. LXIV) becoming dark olive gray in age (Ridgway Pl. LI) in size commonly ranging from 60 to 75 μ in diameter to 200 to 300 μ in length. Conidiophores erect commonly 300 to 400 μ in length by 6.5 to 8 μ in diameter of uniform thickness throughout walls smooth, colorless or very faintly green approximately 1 to 2 μ thick enlarging rather abruptly into an ovate vesicle. Vesicle thin walled variable in form and dimensions but commonly ovoid (fig.

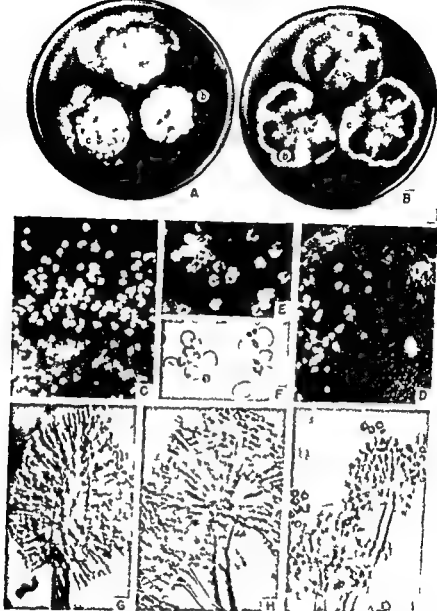


FIG 54 *Aspergillus janus* NRRL No 1787 A and B Colonies on Crapek's solution agar and malt agar respectively showing a scattered white conidial heads, b, crowded green heads and c massed hülle cells two weeks old incubation at 24° C. $\times \frac{1}{2}$ C Characteristic white heads borne on long thin conidiophores $\times 7.5$ D Massed green heads in dense stand adjacent to the substratum with white heads projecting from the left and with localized development of hülle cells in two limited areas at right $\times 7.5$ E Heads of mixed character with older (outer) conidia white and younger (inner) conidia green, $\times \frac{1}{2}$ F Hülle cells $\times 350$ G Typical white head showing clavate vesicle double sterigmata and small smooth conidia $\times 4.0$ H Head of mixed character at this stage (early) producing small white smooth conidia $\times 450$ I Typical green head showing small nearly globose vesicle double sterigmata and larger echinulate green conidia $\times 450$ (Reprinted from Raper and Thom "New Species of *Aspergilli* from Soil" *Mycologia* 36 Nov-Dec 1944)

III I) and occasionally conspicuously elongate typically fertile over the entire area ranging in size from 20 to 30 μ by 12 to 18 μ . Sterigmata in two series rather loosely arranged primaries 7 to 10 μ by 4 to 4.5 μ secondaries 6 to 8.5 μ by 2 to 2.8 μ . Conidia dark green in mass conspicuously spinulose (fig. 54 I) globose mostly 2.5 to 3.5 μ occasionally larger or smaller.

Conidial heads of mixed character containing both white and green spores commonly encountered (fig. 54 E) usually borne upon long conidiophores approaching and often equalling in length those of white heads vesicles clavate (fig. 54 H) sterigmata at first bearing colorless smooth walled conidia but subsequently bearing dark green spinulose conidia. At temperatures of 24° C and above thick walled hülle cells abundant irregular in form (fig. 54 F) commonly globose to subglobose not infrequently elongate commonly more or less curved and often lobed.

Colonies upon malt extract agar growing luxuriantly (fig. 54 B) generally loose in texture with aerial mycelium prominent, conidial heads normally more abundant than upon Czapek agar the proportion of white to green heads varying with the temperature of incubation.

Colonies upon hay infusion agar spreading broadly consisting of a thin submerged mycelium from which develop erect white and green conidial structures the relative proportion of these types being dependent upon the temperature of incubation since comparatively meager growth occurs upon this medium and since there is a minimum of aerial vegetative hyphae it constitutes a very favorable substratum upon which to observe the formation of the contrasting fruiting structures characteristic of the species.

The binomial *Aspergillus janus* was selected for this species because of the contrasting types of conidial heads produced—it is literally a 'two faced' mold.

Type culture NRRL No. 1787 was isolated in February 1942 from Panama soil collected during the summer of 1941 by John T. Bonner of Harvard University. Three additional isolations by members of the Northern Regional Research Laboratory staff have since been made from Panama soils subsequently collected by Mr. Benjamin T. Coghill.

It is believed that this species represents a normal component of the microflora of Panama. Additional evidence in support of this view is furnished by the fact that in 1925 Professor Roland Thaxter sent to Thom under the label white Panama *Aspergillus* a representative of this species. The form was never described by Thaxter and viable cultures of it were lost from our collection some time prior to 1930. As the correspondence of the time is remembered Thaxter was plagued by the presence of a small green mold which repeatedly appeared in his cultures as a 'contaminant'. Fortunately the original tube received from Thaxter has been preserved.

and re examination of this culture leaves no question but that he was dealing with a strain of the species here described and that the green form which troubled him so much was not in fact, a contaminant but a different phase of the same fungus

Aspergillus janus var *brevis* Raper and Thom, in *Mycologia* 36 561-563, fig 2 1944

The variety differs from the species in a number of particulars foremost among which are (1) the reduced length of the conidiophores bearing both white and green heads and (2) a consistent tendency for white and green conidial structures to develop in approximately pure stands and to appear as contrasting radial sectors

White conidial heads are of the same general pattern and form as in the species but are of somewhat smaller dimensions are borne upon conidiophores generally less than 2 mm in length by 6 to 8 μ in diameter and are characterized by elongate but not strongly clavate vesicles measuring 20 to 25 μ by 14 to 18 μ conidia are smooth walled colorless, globose to subglobose, 2.2 to 2.8 μ in diameter Green conidial heads are compact globose to somewhat columnar borne upon conidiophores 75 to 125 μ by 4 to 6 μ with globose to subglobose vesicles measuring 8 to 15 μ by 10 to 18 μ , conidia are dark blue green, strongly echinulate and 3.5 to 4.5 μ in diameter

The vesicles of white heads in the variety *brevis* are of approximately the same size and form as the vesicles of green heads in the species itself, whereas the conidiophores bearing each type of head are approximately one half the length of those bearing the same type of head in the species The most striking character distinguishing the variety, however, is the manner in which areas of white and green heads are sharply separated along radial lines Conidial heads of mixed character are produced and usually can be found along the frontier between white and green sectors

Type culture NRRL No 1935 was isolated in July 1942 from a sample of soil collected in Alameda in southern Mexico and forwarded to us in June by Mr William B Roos

Aspergillus versicolor (Vull.) Tiraboschi in *Ann Botan (Rome)* 7 II 1908

Synonym *S. versicolor* Vuillemin Murski B in *Thèse de Mèd Nancy* no 27 p 15 et seq 1903 See Thom and Church *The Aspergilli* p 142 1926

Colonies upon Czapek's solution agar rather slow growing, compact in some strains velvety and consisting almost entirely of closely crowded conidiophores arising from the substratum in other strains showing a marked development of floccose hyphae bearing more or less abundant

conidiophores as short arial branches in still others a combination of both growth types with colony centers initially floccose and outer areas almost velvety at first white passing through shades of yellow orange yellow, tan

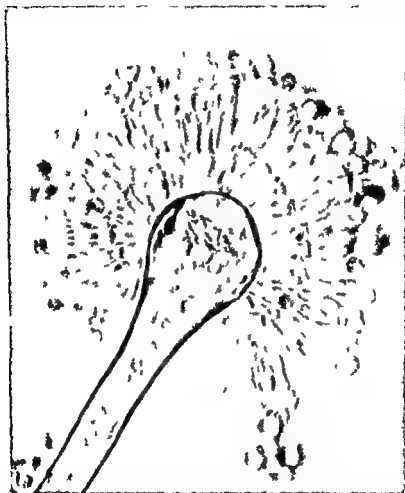


FIG 55 *Aspergillus versicolor* strain NRRL No 2 Photomicrograph showing details of head structure Note particularly the double series of sterigmata $\times 2100$

to yellowish green shades such as pea green or sage green (Ridgway Pl XI V II) depending upon the strain (Pl V C and D and fig 53 C and D) and conditions of culture occasionally with the green colors almost or com

pletely lacking, reverse and substratum occasionally colorless or nearly so, mostly passing through shades of yellow to orange then rose purple red or red, the particular shade and intensity of color normally persisting as a strain or varietal character. Heads roughly hemispherical radiate up to 100 to 125 μ in diameter, rarely approaching columnar. Conidiophores colorless, smooth up to 500 or even 700 μ by 5 μ or approaching 10 μ near the vesicles. Vesicles 12 to 20 μ in diameter, fertile area hemispherical or semi-elliptical (fig 55) passing almost imperceptibly into the funnel like enlarged apex of the conidiophore. Sterigmata in two series primary commonly 8 to 10 μ by 3 μ , occasionally less, secondary 5 to 10 μ by 2 to 2.5 μ . Conidia globose, usually delicately echinulate, mostly 2.5 to 3 μ , occasionally 3.5 μ or 4 μ , usually borne in loosely radiating chains.

In occasional highly colored strains vesicles and sterigmata may be colored.

Hülle cells of the *Aspergillus nidulans* type are occasionally seen.

Neither perithecia nor sclerotia have been found.

The diagnosis is drawn broadly enough to cover a common and very abundant series of organisms which vary greatly in colony appearance. In some strains arial growth is composed of conidiophores and heads only, in others it is made up of floccose felts or ropes of hyphae bearing short conidiophores and small heads which retain the characteristic arrangement of vesicles, sterigmata, and conidial chains.

The wide range of colony structure and coloration of strains,¹ and the varied conditions under which these have been collected probably account for the appearance of many names in the literature which refer to cultures of the group but which cannot be safely separated and identified by the descriptions given.

The following list of species are believed to represent synonyms.

S. ambari Beaugard (Ann de Micrographie 10 255-278 pl 1 1898). Probably one of the series but never identified again.

S. bicolor J Ray (Rev Gen Bot 9 193-212 245-259 292-304 Pl 12-17 1897) is probably one of this series. Primary sterigmata were reported filled with red coloring matter and conidia globose spinulose up to 2.5 μ in diameter.

Sterigmatocystis brodens Mattlet (Name only in Ann Soc Belge Med Trop 6 31 1926 discussion ibid 4 167-171 figs 1 2 1924). Apparently a member of this series.

A. flavo viridescens Hanzawa (Jour Coll Agr Tohoku Imp Univ Sapporo 4 232-3 pl 21 figs 1-4 1911). Culture No 4291 10 (Thom) received from Hanzawa under this name belongs to the *A. versicolor* group.

A. versicolor var *glauca* Blochwitz (Ann Mycol 32(1/2) 86 1934). This variety has the color of the *A. glaucus* group which is more deeply blue than *A. versicolor*. Isolated in the skin clinic at Hazel upon human skin showing ringworm.

¹ For a more complete discussion of the range of cultural types found in this series the reader is referred to Thom and Church 'The Aspergilli' pp 142-145 1926.

S. glauca Bainier (Bul Soc Bot France 27 29-30 pl 1 fig 3 1880 ibid 28 77 1881) From extract of henbane dregs of wine casks and corks

A. globosus Jensen (N Y Cornell Agr Exp Sta Bul 315 p 482 1912) The type culture received from Whetzel (Thom No 2705) is certainly a member of this series and is characterized by yellowish green to olive green conidial areas with colony reverse in yellowish-orange to wine red

S. polychroma Ferraris (Fl Ital Crypt Hyph p 640) Syn *A. versicolor* fide Tiraboschi in Ann de Botanica (Rome) 7 9 1908

S. spuria Schroeter (Cohn Krypto III von Schlesien 3 2 Hälfte Lief 1 p 218 1893) Position in doubt May represent a form of *A. versicolor* similar to the flesh colored forms discussed by Thom and Church in The Aspergilli p 145 1926 or may belong with *A. carneus* (See p 201)

A. tabacinus Nakazawa Sano and Watanabe (Jour Agr Chem Soc Japan 10(2) 177 178 1934) The detailed figures given in contrast to their own strain of *A. versicolor* showed differences which disappear when large numbers of isolations are studied

Aspergillus humicola Chaudhuri and Sachar in Ann Mycol 32 97 1934

Characterization after Chaudhuri and Sachar

Colonies on Czapek's solution agar, at first white passing through shades of olive-gray (Ridgway, Pl XLVI 20-22) velvety at margin floccose toward the center reverse and substratum in shades of yellow, heads radiate Conidiophores arising directly from the substratum up to 300 μ in length by 4 to 5.4 μ in diameter or as short branches, about 70 μ long from serial hyphae walls smooth and almost colorless Vesicles 9 to 15 μ in diameter, colorless flask-shaped with sterigmata radiating from the whole surface of the larger heads or only borne in the upper third in small heads primary sterigmata 3.6 to 5.4 μ by 1.8 to 2 μ secondary sterigmata 3.5 by 1.8 μ Conidia globose smooth 2 to 3 μ in diameter, in radiating chains

Neill (1939) is believed to have correctly placed this organism with *A. versicolor* and its allies despite the smoothness of its conidia The almost colorless conidiophore suggests relationship to *Aspergillus ustus* in this group certain forms (e.g. Blochwitz's *A. ustus* var *laevis*) apparently have spores smooth or nearly so (see p 175)

Pathogenesis

Aspergillus sydowii is not reported by name as a parasite but strains described in terms which must be interpreted as placing them with *A. sydowii* includes *A. funicularis* (Langeron) Dodge from fleshy lesions on a hand in Tunis *A. Vancampenhoutii* (Mattlet) Dodge also from tropical Africa *A. cyaneus* (Mattlet) Dodge from the same region The evidence at hand links *A. sydowii* more closely with *A. nidulans* than was formerly supposed although such relationship is strongly indicated by the identical character of their hülle cells The fragmentary descriptions commonly given for

individual pathogenic molds isolated by persons unfamiliar with the literature are rarely definite enough to separate nearly related forms

An occasional culture of *A. versicolor* is obtained from apparently pathogenic sources. Thus far experimental work has not shown evidence of actual lesions in human flesh. Little colonies bearing green heads and conidia were drawn with a breast pump from an inflamed mammary gland and kept in culture for many years but failed to grow in laboratory media at blood heat. *Aspergillus versicolor* var. *glauca* Blochwitz was isolated from human skin showing 'ringworm' at the skin clinic in Kiel but apparently pathogenicity was not proved experimentally. Strains of *A. versicolor* are frequently observed upon dried salted lean beef thus showing its capacity to grow in and upon meat products, but not giving direct evidence of participation in any pathological process.

Occurrence and Economic Importance

Members of the *Aspergillus versicolor* group appear widely distributed in soil, on spoiling and drying food stuffs breads, cereals, old cheese, dried meats, cured India rubber, musty vegetable products, and other substrata characterized by a moderately low water content or containing factors toxic to most organisms. They are reported as capable of decomposing certain paraffins. The production of proteolytic enzymes by most strains is shown by the digestion of milk and the liquefaction of gelatin. *Aspergillus sydowii*, in particular, is a characteristic component of all soil examined.

Fat production by *A. sydowii* has been studied quite extensively by Professor Peterson and associates at the University of Wisconsin. For reference to this work see the various papers listed in the Topical Bibliography under the heading 'Chemistry of Mold Tissues'.

CHAPTER XV

THE ASPERGILLUS TERREUS GROUP

Outstanding Characters

- Heads columnar in cinnamon pale buff or light flesh colors
 Conidiophores smooth colorless rarely exceeding 250 μ in length
 Vesicles hemispherical with upper half to two thirds covered by sterigmata
 Sterigmata in two series generally crowded
 Conidia smooth globose to slightly elliptical small

Included here are members of a variable and cosmopolitan group of *Aspergilli* especially common in soil. They differ markedly in color and in colony appearance and to a lesser degree in the texture of their conidial heads. The group may be separated as follows:

Group Key

- I Conidial heads in cinnamon or orange brown shades compact uniform in diameter throughout *A. terreus* series
 A Colonies velvety conidiophores mostly in a dense stand arising from the substratum
 1 Conidial heads in dull cinnamon shades *A. terreus* Thom
 2 Conidial heads orange brown near xanthine orange (Ridgway)
 A. terreus var *boedijnii* (Bloch) n var
 B Colonies floccose conidial heads arising from aerial hyphae
 1 Mycelium colorless heads light pinkish-cinnamon in color
 A. terreus var *floccosus* Shih
 2 Mycelium yellow heads developing late in cream or light tan shades
 A. terreus var *aureus* n var
- II Conidial heads white or flesh colored loose textured not strictly uniform in diameter *A. carneus* series
 A Colonies in light flesh colors ranging from near white to vinaceous fawn (Ridgway). Thick walled hyphae suggestive of hülle cells are generally present *A. carneus* (van Tieghem) Blochwitz
 B Colonies persistently white or becoming dull ivory in age. Thick walled cells often present *A. niveus* Bloch

Aspergillus terreus Thom in Turesson Gote Svensk Botanisk Tidskrift
 10 5 1916 without description diagnosis Thom and Church
 in Amer Jour Bot 5 85-6 1918

Synonym *A. galentius* Blochwitz in Ann Mycol 27(3/4) 205 Taf
 III 1929

Colonies upon Czapek's solution agar growing well at room temperature and up to 37 C spreading plane or marked by shallow radial furrows



FIG. 56 *Aspergillus terreus*. A Colony margin of a typical strain NRRL No. 265 showing crowded long columnar heads $\times 118$. B Scattered but typical conidial heads of an ultra violet light produced mutation unable to utilize NO_3 nitrogen $\times 60$. Both cultures on Czapek's solution agar 2 weeks.

velvety (fig 58 A) or in some strains showing tendency toward floccosity in central colony areas heavy sporing throughout with massed columnar heads giving to colonies their characteristic color and texture, in color ranging through various cinnamon shades (Ridgway, Pl XXIX) depending upon the abundance and maturity of the heads (Pl V E) amber exudate produced in some strains odor transient to none reverse in dull yellow to brown shades Conidial heads long columnar (fig 56 A) with conidial chains compacted together of uniform diameter throughout entire length commonly ranging from 150 to 500 μ or more in length by 30 to 50 μ at maturity, ranging from cinnamon buff through cinnamon to Sayal brown (Ridgway Pl XXIX) Conidiophores more or less flexuous smooth, colorless commonly ranging from 100 to 250 μ by 4.5 to 6.0 μ approximately uniform in width throughout (fig 57 A and 19 A) Vesicles hemispherical dome like commonly 10 to 16 μ in diameter merging almost imperceptibly into the supporting conidiophore Stigmata in two series primaries crowded (fig 57 A) parallel 5.0 to 7.0 μ by 2.0 to 2.5 μ secondaries closely packed 5.5 to 7.5 μ by 1.5 to 2.0 μ Conidia globose to slightly elliptical commonly 1.8 to 2.4 μ in diameter

Species description based upon type strain NRRL No 255 (Thom No 144) and innumerable additional isolations from soils and other sources in this country and abroad The species is especially abundant in warm and comparatively dry arable soils

The great majority of isolates belonging to this series fall within *A. terreus* in its strictest sense and duplicate in all essential particulars the description given above for this species Nevertheless wide natural variation among strains is encountered when great numbers are isolated from widely separated sources Some of these are quite striking in appearance and have apparently furnished the bases for species and varietal description by other workers

Aspergillus terreus var *boedijnii* (Bloch) = var

Blochwitz in Ann Mycol 32(1/2) 83 1934 described *Aspergillus boedijnii* as a new species differing from *Aspergillus terreus* primarily in the color of its conidia The were reported as pure yellow at first becoming pure brown or ochraceous brown in age and brighter than *A. galeritus* Blochwitz¹ In our experience strains are occasionally encountered which are characterized by a bright orange brown color instead of the dull cinna

¹ *A. galeritus* Blochwitz (Ann Mycol 27(3/4) 205 Taf III 1929) is a redescription of *A. terreus* Thom Blochwitz acknowledged having Thom's type at hand when he renamed this species No reason was given beyond the claim that he had had the organism in culture for some years before Thom's description of *A. terreus* was published

mon shades typical of the species. Among cultures currently under examination, this character is noted in an isolate from Argentine soil is somewhat more marked in NRRL No 680 from Dr G A Ledingham Ottawa, Canada and is particularly striking in NRRL No 1913 isolated by Dr C W Emmons from Arizona soil. In all of these strains the basic morphology is that of a typical *A. terreus* hence Blochwitz's separation is reduced to varietal rank.

Aspergillus terreus var *floccosus* Shih, in Langnan Sci Jour 15 372, Pl 16 fig 3 1936

Strains characterized by deep floccose colonies in which conidial heads are less abundant develop late and are borne almost entirely upon aerial hyphae are frequently encountered (fig 58 B). While the conidial structures of certain of these strains appear entirely normal in the majority of isolates the heads are somewhat less compact and generally lighter in color. This color difference is particularly marked among isolates from soils collected in Texas Central America and Cuba with color commonly light pinkish-cinnamon (Ridgway Pl XLIX) to vinaceous buff (Pl XL) in age. No sharp line of separation can be drawn between typical strains of *A. terreus* and the floccose forms under consideration since isolates of intermediate character are encountered, nevertheless these strongly floccose cultures occur with sufficient frequency to warrant recognition of Shih's varietal designation if his interpretation is somewhat broadened. The variety is considered by the writers as a strongly floccose *Aspergillus terreus* in which the head is commonly less compact and lighter in color, but with the basic morphology of the conidial apparatus remaining that of the species proper. This variety is represented by such strains as NRRL Nos 1920 and 1921 isolated from Cuban soil contributed by Professor J M Osorio University of Havana, No 1922, isolated from Texas soil collected and sent to us by Dr F E Clark from Greenville Texas.

Other strains examined almost completely bridge the gap between the pale colored strains of *A. terreus* var *floccosus* and the light flesh colored forms characteristic of *Aspergillus carneus*.

Aspergillus terreus var *aureus* n. var

This new and striking variety differs from the species in a number of particulars. Colonies upon Czapek's solution and malt extract agars are comparatively slow growing, floccose, ranging up to 3 to 4 mm deep, and are bright golden yellow in color. Conidial structures are produced tardily and in limited numbers. Conidiophores are appreciably longer than those of the species often becoming 500 μ or more in length and bear columnar heads generally loose in texture ranging in color from cream or light buff

to light pinkish-cinnamon. Microscopically the conidial structures approximate those of the species itself. Separation as a new variety is based primarily upon the characteristic coloration of the growing colony.

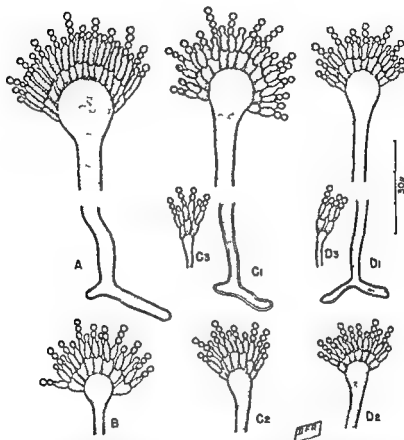


FIG. 57. Conidial structures of members of the *Aspergillus terreus* group. $\times 840$. A, *A. terreus* type strain NRRL No. 255 (Thom No. 164). B, *A. terreus* var. *aureus* NRRL No. 1923. C₁, C₂, and C₃, *A. carneus* NRRL No. 1923, conidial heads vary greatly in size. D₁, D₂, and D₃, *A. niger* NRRL No. 515, conidial heads of varying dimensions.

Type strain NRRL No. 1923 (fig. 16 D) was isolated from Texas soil contributed by Dr. F. E. Clark. Additional strains showing approximately the same cultural and morphological characteristics have been isolated from soils collected in Arkansas and Arizona. In *A. terreus* var. *aureus* the yellow coloring matter is lodged in the vegetative mycelium and there are no suggestions of hülle cells. In *A. carneus* however approximately the same

yellow tints are developed through the massing in localized colony areas of thick walled hyphae suggestive of hülle cells (see p 201)

At least two other species have been described which are believed to represent probably synonyms of *Aspergillus terreus*

Aspergillus fuscus Amons (Archief voor de Suikerindustrie in Nederlandsch Indie Jaarg 29 Deell pp 8-10 1921) by description is obviously a form closely related to if not identical with *A terreus*

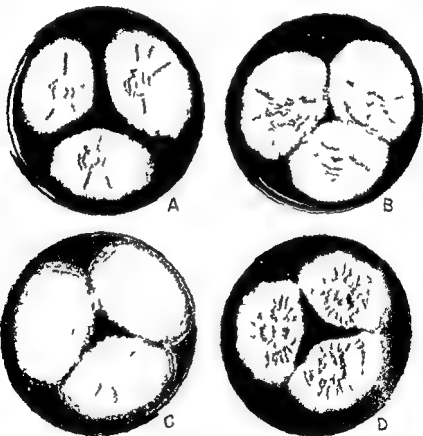


FIG 58 *Aspergillus terreus* group different species growing upon Czapek's solution agar at room temperature A Typical, heavy sporing strain of *A terreus* NRRL No 265 B *A terreus* var *floccosus* characterized by loose floccose colonies and limited spore production C *A carneus* NRRL No 1923 heavy sporing and characterized by flesh colored conidia D *A nireus* NRRL No 515 characterized by white conidial heads

Aspergillus cinnamominus (Weiss) Dodge in Med Myc p 677 1935

synonym *S cinnamominus* Weiss in Ann Parasitol Hum Comp 8 189-193
5 figs 1930

Characterization from Dodge Hyphae septate and branched conidiophores simple 5µ in diameter vesicle 12 by 9µ Primary phialides cylindric 4µ long bearing

2 to 3 sometimes 4 secondary phialides 5 to 6 μ long. Conidia spherical 1 to 2 μ in diameter brown. Other spores borne laterally on short branches (phialides?) 3 to 4 μ . Chlamydospores occasional. Under some conditions only monstrous phialides are formed with a pleomorphic mycelium resulting. Described as present in lesions of pityriasis versicolor flava. Inoculation into the human skin reproduced the original disease. From the description given the organism is some strain of the *A. terreus* group.

Aspergillus carneus (v. Tiegh) Blochwitz

Synonyms *Sterigmatocystis carnea* v. Tiegh in Bull. Soc. Bot. France 24: 103, 1877. Cited also in Saccardo Sylloge 4: 74 and in Wehmer's Monograph p. 127 (Mem. Soc. Phys. Hist. Nat. Gen. pp. 1-157, 1899-1901).

Sterigmatocystis spuria Schröter as a bibliographic change in Cohn Krypt. Fl. Schleisen 3: 2, Hälfte Lief. 1 p. 218, 1893.

Aspergillus carneus Blochwitz in Ann. Mycol. 31(1/2): 81, 1933.

Colonies upon Czapek's solution agar growing well at room temperature spreading plane or radially furrowed more or less floccose at first white but becoming pale vinaceous fawn to vinaceous fawn (Ridgway Pl. XL) with the development of mature fruiting structures (Pl. V F and fig. 58 C) ranging from 1 to 2 mm or more deep in central areas to very thin and spreading at colony margin comparatively heavy sporing throughout with fructifications arising from both aërial and submerged mycelium some strains showing limited areas yellow in color from an underlying felt of heavy walled sterile hyphae suggestive of hülle cells (fig. 49 F) odor often pronounced somewhat putrid reverse in orange-yellow bright orange, to deep brown shades conidial heads loosely columnar averaging 150 to 200 μ by 25 to 35 μ but commonly somewhat larger varying in color as the colony. Conidiophores variable in length mostly 250 to 400 μ but ranging up to 1 mm occasionally bearing secondary fruiting structures as short and irregularly placed branches smooth sinuous uncolored mostly 3.5 to 6.0 μ in diameter. Vesicles hemispherical, ranging from 5.5 to 9 μ rarely as much as 10 μ (fig. 57 C₁). Sterigmata in two series, primary 5.5 to 6 μ by 2 to 2.5 μ secondary 5 to 5.5 μ by 1.8 to 2 μ commonly very few primary sterigmata present. Conidia globose to subglobose thin walled averaging 2.4 to 2.8 μ with maximum rarely exceeding 3.2 μ .

Colonies upon malt extract agar growing more restrictedly heavier sporing with pigmentation generally more pronounced and with conidial heads averaging slightly larger than on Czapek's solution agar otherwise duplicating the above description.

This species is represented by strains NRRL No. 527 isolated as an air contaminant in Washington NRRL No. 298 isolated from Kansas soil, and other soil isolations from different parts of the United States Mexico

Cuba, and Central America. Strains differing from the above in the absence of any yellow, thick walled hulle like cells are occasionally isolated from soils. NRRL No 1928, isolated from Arkansas soil, is representative. In the absence of any yellow component, the color of these strains is more accurately described as pale to light grayish vinaceous fawn (Ridgway, Pl XXXIX).

The name *Aspergillus carneus* is revived to cover the forms under consideration since their most obvious identifying characteristic is the pale flesh color of their massed conidial heads. It is the belief of the writers that van Tieghem probably had in hand some member of this series when he proposed the name *S. carnea*, although due to the inadequacy of his description it is now impossible to establish this point with certainty. In any case, the name is excellently descriptive of strains commonly encountered, hence its application in this connection.

In describing *Aspergillus carneus* as a new species, Blochwitz (1933) acknowledged the earlier use of this specific name by van Tieghem but disregarded its validity. He undoubtedly applied it to a member of the species as it is considered by us since he noted that it differed from *A. terreus* (*A. galertius*) principally in the flesh to rose color of its conidia. It is believed that Blochwitz's *A. nucus* var *nubila* (1934) likewise represented a strain of *A. carneus* characterized primarily by conidia of darker rose a condition which in older cultures is frequently suggested by NRRL No 1928 cited above.

Gilman and Abbott in their 'Summary of Soil Fungi' (1927) called attention to the repeated isolation from Louisiana soils, of forms with the 'general morphology of the *Aspergillus candidus* group but producing bright pink conidial heads'. While the writers think the affinities of these forms lie more with *Aspergillus terreus* (columnar heads, colorless conidiophores) and *Aspergillus flavipes* (elongate irregular hulle cells) than *A. candidus* we have every reason to believe they were dealing with forms similar to those here designated *A. carneus*.

S. albo rosea Sartory, Sartory and Meyer (Ann Mycol 28 353-359 Pl III fig 1-6 1930) apparently represents a member of this series. This is indicated by the described coloration of colonies and more particularly by the detailed measurements cited for it.

Aspergillus nucus Blochwitz, in Ann Mycol 27(3/4) 203-6 fig 2
Tab III 1929

Synonym *A. eburneus* Bourge name attached to a culture received by Thom (No 5402 1 NRRL No 515)

Colonies upon Czapek's solution agar white, plane or radially furrowed rather slow growing forming a dense felt of mycelium and conidiophores up

to 600 μ to 1 mm deep thinning toward the margin (fig 58 D) and commonly spreading in unevenly radiating lines commonly producing abundant amber to brown exudate reverse in dark yellow shades through greenish to brownish black odor slight Conidiophores smooth with walls colorless sinuate more or less septate slender 4 to 6 μ in diameter, enlarging to a hemispherical vesicle 8 to 15 μ in diameter or sometimes larger at the apex commonly 300 to 600 μ in length occasionally up to 1000 μ long and on other substrata sometimes longer Conidial heads showing chains of conidia in comparatively loose columns most frequently 20 to 30 μ in diameter but in large heads up to 60 μ with the general appearance of a snow white *A. terreus* Vesicular area hemispherical (fig 57 D) Sterigmata in two series primary sterigmata 5 to 8 μ by 2.5 to 3.0 μ secondary sterigmata 5 to 7 μ by 2 to 2.5 μ Conidia 2 to 2.5 μ rarely more smooth thin walled colorless

Represented in the NRRL collection by Nos 515 (Thom No 54021) 288 and 1955 Repeatedly isolated from soil but less common than *Aspergillus carneus* and the ubiquitous *A. terreus*

Typically the conidial apparatus is that of a white loosely columnar form of *A. terreus* As described by Blochwitz it does not show yellow in culture This is true of strain NRRL No 515 although in age this culture reaches dull ivory to pale buff on agar slants In other strains such as NRRL No 1955 from Dr Timonin in Ottawa Canada limited areas may become yellow from the development of massed thick walled hyphae as in *A. carneus*

Sterigmatocystis pusilla Peyronel (I germi atmosferici dei funghi con micelio Thesis Padova 1931 p 21) probably represents a synonym of *A. niseus* Blochwitz It was described as follows Colonies white very thin sterile hyphae creeping sparingly branched falsely septate hyaline 1.5 to 5 μ in diameter conidiophores erect unseptate hyaline 60 to 80 μ by 2.5 to 3 μ with apical vesicles hyaline obovoid or subglobose 7 to 10 μ in diameter sterigmata radiate in two series with primaries 5 to 7 μ by 2.5 to 2.5 μ and secondaries 3 to 5 μ by 2 μ in groups of 2 to 3 conidia globose 2 to 2.5 μ hyaline smooth Habitat from air in northern Italy at altitude of 1700 meters From description this would appear to represent a short stalked member of *A. niseus*

In their most typical manifestation the conidial heads of *Aspergillus niseus* are loosely columnar vesicles are dome like and fertile over the upper one half to two thirds only They thus stand out in sharp contrast against the typically globose heads and completely fertile vesicles of *A. candidus* But all heads of *A. candidus* are not globose and all vesicles are not fertile over their entire surface In most strains small columnar heads not particularly different from those of *A. niseus* can be found (fig 60 D) Considering this character together with (1) the snow white heads (2) the smooth colorless conidiophores and (3) the small smooth conidia of both species one can readily imagine that we are here dealing with two interlock

ing groups. One basic character separating the two is the production of sclerotia. Characteristic reddish purple to black sclerotia are commonly found in white spored strains producing wholly or in part large globose heads (*A. candidus*), they have never been seen in white spored strains producing only loose columnar heads (*A. niger*). Until additional intermediate forms are isolated and studied, the relationship between these white spored forms must remain a matter of conjecture, although in this manual they are placed adjacent in what we believe to represent a natural placement of the different groups.

Pathogenesis

Strains of *A. terreus* grow under a wide range of temperature including 37° C. It is not surprising, therefore, that an occasional member of the series is reported as a human parasite. One of these was re-described as *Sterigmatocystis hortai* by Langeron (1922). This culture, NRRL No. 274 (Thom No. 5071 1), received from France, was originally isolated from a human ear in Brazil. It is believed to be type and represents a characteristic strain of *A. terreus*. Another was found in metastatic lesions on a corn husker's hand and forearm in Nebraska. Recently a strain was isolated from an aborted fetus from a cow in Maryland, the culture was entirely typical of *A. terreus*.

Occurrence and Economic Importance

Members of the *Aspergillus terreus* group are typically soil organisms, hence are most abundant in soil and upon decaying vegetation. They frequently occur however upon a great variety of materials useful to man, including grains in storage, straw and forage products, cotton and other fibrous materials not adequately protected from excessive moisture, etc. *Aspergillus terreus* and *Aspergillus carneus* are especially widespread in warm arable soils and have been isolated in great abundance from soils collected in southern and southwestern United States. They are generally less common in forest than in cultivated soils and are rarely found in acid forest soils from the colder temperate zone. There is little evidence that these forms are especially active agents of decay, but their great abundance in nature indicates that they undoubtedly play a significant role in the slow decomposition of organic materials. *Aspergillus terreus* and *A. carneus* grow well at temperatures of 35 to 37° C., a character which possibly accounts for their great abundance in southern soils and their relative scarcity in soils from northern areas.

Aspergillus terreus has become of special biochemical interest since the discovery in 1939 by Calam, Oxford and Raistrick that certain strains of this species are capable of producing itaconic acid from sugars. Extensive

investigations on this fermentation have been conducted in the Fermentation Division of the Northern Regional Research Laboratory, and papers reporting these studies are currently in press. More than 300 strains have been tested and the most productive cultures selected for intensive study. Improved nutrient solutions have been developed and critical environmental factors explored with the result that substantial yields of itaconic acid are now obtained. It is believed that this process may become of industrial importance within a reasonable period of time. (See Lockwood Raper, Moyer, and Coghill; Lockwood and Reeves; Lockwood and Ward, Moyer and Coghill, and Raper; Coghill and Hollaender, in the *Topical Bibliography* under 'Itaconic Acid'.)

Timonin (1942) reported the production of citrinin by a white-spored *Aspergillus* identified by him as one of the *A. candidus* group. Careful examination and comparison of his culture with representative strains of *A. candidus*, *A. carneus*, and *A. niger* show its true relationship to be with *A. niger* in the *terreus* group as it is considered here rather than with *A. candidus*.

CHAPTER XVI

THE ASPERGILLUS CANDIDUS GROUP

Outstanding Characters

- Conidial heads persistently white or becoming yellowish cream in age, typically globose but approaching columnar in small heads
- Conidiophores smooth, colorless or slightly yellowed in terminal areas
- Sterigmata in two series with primaries often much enlarged sometimes varying greatly in size within the same head
- Conidia globose or subglobose smooth
- Sclerotia present in some strains dark, approaching purple to black when mature

Grouping by color led Thom and Church (1926) to establish their Section IX or the so-called "White spored Aspergilli." Only vaguely did they indicate that they had included a heterogeneous lot rather than described a natural group. Further study of the strains included in the 'white' section showed the tardy development of colors approaching avellaneous or even carneus. The continued comparison of great numbers of strains in all groups has revealed such wide variations in color that the authors have come to regard whiteness or lack of color, as a character of secondary importance in the allocation of strains to particular groups. This view point is supported by the appearance under controlled conditions of white variants or mutants in a number of colored series. Yuill (1939) observed and isolated such colorless mutants from *Aspergillus fumigatus* and *A. nidulans*, Steinberg and Thom (1940) reported the same type of mutant for the former species, while Raper, Coghill and Hollaender (in press) have succeeded in producing white mutants in *Aspergillus terreus* by irradiating spores with ultra violet. Colorless members of the *Aspergillus glaucus* group represented by *A. niteo-glaucus* have been isolated by Blochwitz. Thom and Raper and other investigators. Long before the work of Yuill Schiemann (1916) had developed two color variants of *Aspergillus niger* *A. schiemanii* (Schiemann) Thom (1926 p. 172) and *A. cinnamomeus* Schiemann (1912) which differed only from the parent strain by a progressive reduction of the amount of coloring substance, presumably the aspergilline of Lanossier (1891). Steinberg and Thom (1940) working with a strain of *A. niger* again produced variants approximating those of Schiemann and Whelden (1940) secured the same by irradiating spores of *A. niger* with cathode rays. In cultures

collected from nature from world wide sources strains characterized by heads approaching white are occasionally observed in other groups. It is apparent then that the capacity to produce a coloring substance while ordinarily inherited or passed on in successive colonies of an organism is not always uniformly maintained.

In the present treatment the writers have sought to include within the *Aspergillus candidus* group only such forms as are clearly and closely related to it. The group is thus limited essentially to a single series containing only one clearly definable species *A. candidus*. Different isolations vary materially in their general cultural appearance and in the details of their microscopic structure. Nevertheless all possess the typically globose white to dull buff or light gray conidial heads, the smooth colorless conidiophores and the small smooth colorless conidia.

To facilitate recognition of members of the *Aspergillus candidus* group and to assist in the proper assignment of other white or light-colored species and strains a general key covering all of these forms is presented.

GENERAL KEY OF WHITE ASPERGILLI

- A Heads (large ones) globose or radiate conidiophores smooth walled colorless or yellowed toward the vesicle only sclerotia occasionally seen *A. candidus* group
- B Heads white hemispherical to columnar conidiophores smooth walled colorless
A. niger series see p. 202
- C Heads initially white tending to be columnar conidiophores smooth walled showing some shade of yellow contorted hülle cells usually found
A. flavipes series see p. 179
- D Heads white borne upon long smooth walled colorless conidiophores terminating in clavate vesicles sterigmata in two series
White-spored phase of *A. janus* see p. 187
- E Strains of white Aspergilli possessing the basic characters of their colored counterparts also occur as mutations in the *A. fumigatus*, *A. nidulans*, *A. terreus* and *glaucus* groups

Aspergillus candidus Link. Obs. p. 16. 1809. Thom and Church. The Aspergilli. p. 157. 1926.

Colonies upon Czapek's solution agar persistently white or becoming cream or yellowish-cream in age (Pl. VI A and fig. 59) often thin vegetative mycelium often largely submerged surface growth usually consisting of conidiophores and heads and with scanty sterile mycelium or anastomosing ropes of hyphae bearing short stalked fruiting structures sclerotia produced in occasional strains reverse usually uncolored. Heads white globose radiate varying in the same culture from large globose masses 200 to 300 μ in diameter to small heads often less than 100 μ in diameter commonly more or less elongated in heads with incomplete development of sterigmatic surface. Conidiophores varying with the strain

in short or dwarf races less than 500μ long, in other strains ranging up to 500 to 1000μ or longer, varying from 5μ in diameter in dwarf forms to 10 to 20μ in long stalked forms, with walls thick, smooth, colorless or slightly yellowed near the vesicle in certain strains in age. Vesicles typically

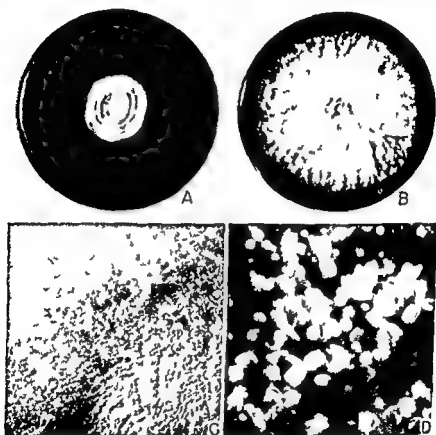
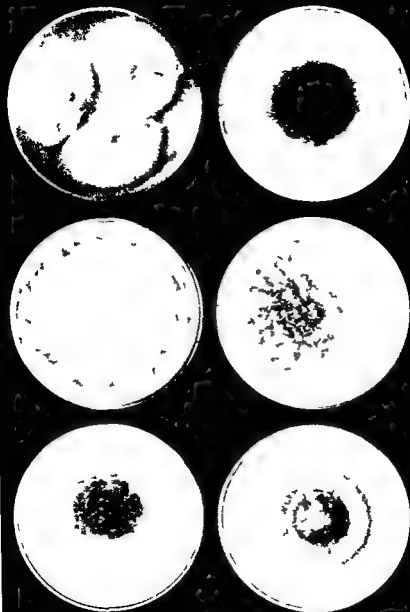


FIG 59 *Aspergillus candidus*. A Strain NRRL No 305 on Czapek's solution agar at room temperature 10 days. B Strain NRRL No 314 on Czapek's solution agar at room temperature three weeks. Note contrast between compact colony of No 305 consisting of crowded conidiophores and loose spreading colony of No 314 in which production of conidial heads is very irregular. C Strain NRRL No 312 portion of colony showing scattered black sclerotia $\times 3$. D Strain NRRL No 308 conidial heads $\times 11$.

globose, ranging from 40μ in diameter in very large heads (fig 60 A), and typically fertile over the whole surface to small globose heads (fig 60 B) often very much reduced to support simple groups of sterigmata appearing almost penicillate (fig 60 D). Sterigmata typically in two series, usually colorless primary varying greatly in different strains in different heads of



P VI

A (type left) A per H ca d d I k NRRL N 308 B (type right) A per H per g D
 ra NRRL N 6 C (see table 1) Y or red m f in 6 p od ced by 1 ra 1 t rad
 D (see table 1) A per H th NRRL N 51 E 1 w 1 ft) A per H alluvu Tl m
 d Ch II NRRL N 315 F G n ft A per H or 1 W b NRRL N 35 All it res

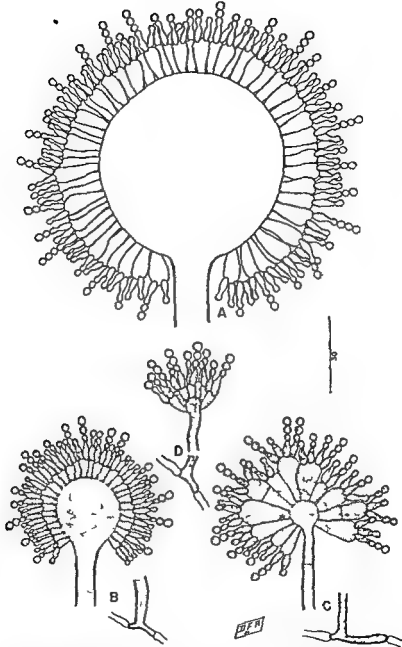


FIG 60 Comdial structure of *Aspergillus candidus* X 900 A Large globose head showing large primary sterigmata strain NRRL No 312 B, Smaller globose head showing small primary sterigmata strain NRRL No 308 C Head showing primary sterigmata of variable size some septate strain NRRL No 312 D Diminutive head same strain Great variation in dimension is characteristic of the fruiting apparatus of most *A. candidus* strains

the same strain, and occasionally in the same head (fig 60 C) ranging from 5μ in length in some cases to 15 or 20μ and even 30μ under other conditions commonly septate secondary sterigmata usually uniform in all heads from 5 to 8μ by 2 to 2.5 or 3μ . Conidia colorless globose or subglobose in most strains to elliptical or barrel form in others, thin walled 2.5 to 3.5μ or occasionally 4μ smooth

Reddish purple to black sclerotia, consisting of thick walled, parenchyma like cells, occur in many strains (fig 59 C)

As there is no possibility of determining which of the white *Aspergilli* was in Link's possession, the name is here used to cover a whole series of strains which are found everywhere but are most frequent in the later stages of decay in vegetation and are especially characteristic of moldy grain. Included within the series as we consider it are two rather different cultural entities. The first of these is characterized by thin colonies in which the mycelium is largely submerged and only fruiting structures, commonly arranged in concentric zones, rise above the level of the substratum (fig 59 B). In other strains colonies are rather floccose and somewhat felted and often attain a depth of 2 mm with fruiting structures arising from aerial as well as submerged mycelia. Sclerotia generally in purple or black shades regularly and consistently develop in many strains including representatives of both of the above colony types. Conidiophores vary greatly in size and characteristically reach their greatest dimensions upon the drier portion of agar slants, or upon slightly moistened grain and other comparable products low in water content. The sterigmata in organisms of this group warrant particular attention since the two series commonly but not consistently differ tremendously in size. In many strains the primary sterigmata are characteristically wedge shaped and reach dimensions of 25 to 30μ by 10 to 12μ (fig 60 A and C) such structures are commonly septate. In other heads from the same culture the primary sterigmata may be relatively small and measure 6 to 8μ by 2.5 to 3.5μ (fig 60 B). Secondary sterigmata are consistently small and of the dimensions indicated in the species description.

'White' *Aspergilli* regularly constitute a normal element in the micro population from moist or improperly dried grains and of comparatively dry vegetation undergoing slow decay. From such material many investigators have described molds characterized by white heads which obviously belong in this group but without supplying sufficient critical data to permit subsequent verification of the exact types under study. Some of these descriptions were based upon molds growing in culture more of them were not. A few of the more tangle of these probable synonyms will be briefly considered in this connection, others will be found in the general species index (pp 331-7)

S. alba Bainier (Bull. Soc. Bot. France 27: 30, 1880) was isolated from oatmeal and while incompletely described obviously represents a member of the *A. candidus* series. Several publications present elaborate comparative tables to separate strains accepted as *A. candidus* and *A. albus* but the many strains obtainable vary into each other so completely that little or no basis for separation exists in fact.

A. albus Wilhelm (Beitr. z. Kenntn. d. Pilzgattung *Aspergillus* Inag. Diss. Strassburg p. 11, 1877) was described with characters which clearly ally it with *A. candidus* but without sufficient differences to separate it from other members of this group.

S. blanc jaune Bainier nomen nudum—A culture from Bainier's collection received by Thom under this name (No. 4640-490) represents a somewhat diminutive but otherwise typical member of this series.

S. albo lutea Sartory and Meyer (Cited by Blochwitz in Ann. Mycol. III 73, 1933). Conidia were reported as turning yellowish in age. This character is common to many members of the group and has been so noted by Wehmer (1889-1901), Thom and Church (1906) and others. Retention of the species name is not warranted.

A. basidioclepta Sartory Sartory and Meyer (Ann. Mycol. III 317-320 Pl. 7, 1929) apparently represents a member of the *A. candidus* series with comparatively long (28 to 30 μ) primary sterigmata which in age are characteristically septate. This character which appears also in some members of the *A. niger* and *A. ochraceus* groups however is not sufficiently unique to warrant specific separation.

A. niseus var. *major* Blochwitz (Ann. Mycol. 32(1/3): 66, 1934). Described as showing vesicles globose, rarely oboval or pear shaped, entirely covered with radiating sterigmata which are rarely absent toward the base, closely growing conidiophores 2 to 2.5 mm. high. These characters suggest relationship with *A. candidus* rather than *A. niseus*.

A. oka-aki: Okazaki in Centralb. f. Bakt. etc. 2 abt. 19 p. 431-431 taf. I, 1907; see also Centralb. f. Bakt. etc. 2 abt. 42 p. 225, 1914. This is cited by Saccardo in Syll. 22, 1960, 1913 as *S. oka-aki*: Saito but apparently without adequate ground for attributing the name or description to Saito.

Colonies described as white to sulphur yellow, conidiophores hyaline, straight or sinuate, smooth or asperulate, 200 to 500 μ by 8 to 12 μ , figured as undulate, especially toward the base, with walls 2 to 3 μ thick, heads 60 to 100 μ in diameter, vesicles 12 to 40 μ in diameter, primary sterigmata 15 to 30 μ by 6 to 8 μ , secondary 8 to 14 μ by 2.5 to 4 μ , conidia globose, hyaline, 2.5 to 5.4 μ , smooth with connectives. In the event that continued study of these white forms reveals the existence in nature of strains with more or less roughened conidiophores and conidial heads ranging to yellow, recognition of *A. okazaki* as a separate species would be warranted. Based upon current information, however, we believe it preferable to consider it synonymous with *A. candidus* Link.

A. sachari of Chaudhuri and Sachar (Ann. Mycol. 32: 95, 1934) is more or less arbitrarily left where the authors put it—as one of the 4 *sulphureus* series near *A. quercinus* in the *A. ochraceus* group. The heads are pale yellow, the sclerotia are near the colors of that group but the conidiophore is described as colorless and smooth which would put it in *A. candidus*.

A. sterigmatophorus Saccardo in Mycologica Venetiae Specimen. Atti d. Soc. Ven. Trent. d. Sci. Nat. 2 fasc. 2: 232, Tab. XVII fig. 5-8, 1873. Syn. *S. italica* Sacc. in *■ italici* no. 109, 1881, changed to *S. italica* Sacc. as a note only in Michelia 1: 91, 1877. Latin diagnosis of *S. italica* in Saccardo Sylloge 4: 72, 1886. Described from decaying corn kernels (*Zea mays*) white, sparse with conidiophores unbranched, 2 to *■* septate above with vesicles globose, sterigmata described as dichotomously or trichotomously branched with ultimate cells bearing conidial chains.

conidia globose about 6μ in diameter with connectives. The description repeats observations as to occurrence and appearance that are frequently seen. No one has since reported a member of the *A. candidus* group with conidia 6μ in diameter. Whether the organism described by Saccardo was a large spored mutant not now isolated remains open to question.

OTHER WHITE ASPERGILLI

Other *Aspergilli* characterized by white heads but differing basically in morphology from the *A. candidus* group occur in the *Aspergillus glaucus*, *A. nidulans*, *A. fumigatus*, and *A. terreus* groups. Except for an absence of spore color these duplicate the morphology of the groups to which assigned. In fact in all cases except that of *A. nuceo-glaucus* in the *A. glaucus* group, they represent colorless mutations produced experimentally from typical parent strains (Yuill, 1939, Sternberg and Thom, 1940, Raper, Coghill, and Hollaender, in press). While *A. nuceo-glaucus* was isolated from nature and hence its parentage is not known, it is suspected that this represents a mutation of some form close to *Aspergillus echinulatus*. *A. halophilus* of Sartory et al (Ann Mycol 28 (3/4) pp 362-3 Pl 3, 1930) similarly belongs in the *A. glaucus* group. Attention has been called earlier to the fact that *A. candidus* differs from *A. niger* primarily in the absence of color and in possessing smooth spores. The question may arise whether we are not here dealing with a whole series of mutations from colored forms. While this is possible, no proof is at hand. The fact that they constitute such a typical and abundant element of the micropopulation of soil decaying vegetation, etc., demands that they be considered along with other major groups of the *Aspergilli* quite aside from any questions of possible origin.

Sclerotia

In this arrangement of the *Aspergilli* sclerotia, as compact globose or subglobose bodies composed of thick walled pseudo parenchyma are not found in the groups characterized by the production of perithecia and ascospores and only rarely, if at all in groups characterized by the presence of hülle cells. In the great groups beginning with *A. candidus*, sclerotia appear with sufficient frequency to be morphologically significant as indicative of class relationship. Fundamentally the typical *A. candidus* strain differs little from the black *Aspergilli* except for the absence of the dark color and rough spores.

Group Relationships

While there is much evidence of relationship with the black *Aspergilli* (smooth walled conidiophores globose vesicles and heads and the presence of sclerotia) there are also certain indications of relationship to *Aspergillus nuceus*. Typically both are characterized by snow white conidia

heads and in all strains of *Aspergillus candidus* there are more or less abundant small heads which bear few and loosely arranged sterigmata in a manner strongly suggestive of typical conidial structures of *A. niger*. Although it is our belief that these similarities in structure do not of necessity reflect close relationship between *A. candidus* and *A. niger*, we do feel that there is need for additional study of strains which appear to be more or less transitional between the two groups.

Occurrence and Economic Importance

Members of the *Aspergillus candidus* group are very widely distributed in nature and occur with reasonable frequency upon vegetation in the later stages of decay. They are especially common upon moldy grains and are obviously able to grow in the presence of a very limited amount of moisture.

The biochemical and physiological activities of these forms have not been studied extensively. *A. okazaki* was employed by Okazaki (1907 and 1914) for the production of a proteolytic enzyme preparation, digestin, and is the basis of a Japanese patent No. 11461 covering this process. Recently Timonin (1942) has employed a strain reported as belonging to the *A. candidus* group for the production of citrinin. Upon examination, however, this strain is found more nearly to represent *A. niger* than *A. candidus* in the sense it is considered here.

- II Sterigmata in one series (Secondary sterigmata occasional in some strains)
- Aspergillus luchuensis* series
- A Colonies black or black brown
- 1 Sterigmata 6 by 3μ (very short) *A. luchuensis* Inui
- 2 Sterigmata about 15 to 20μ conidia 3 to 3.5μ *A. nanus* Montagne
and/or *A. subfuscus* Johan Olsen
- B Colonies in reddish brown shades
- 1 Conidia globose *A. japonicus* Saito
- 2 Conidia elliptical *A. violaceo fuscus* Gasperi

Because of their great abundance in nature, the series most closely related to and including van Tieghem's species based upon strains which satisfy his original description in a somewhat broadened sense will be discussed first.

ASPERGILLUS NIGER SERIES

Species Characterized by Comparatively Small Primary Sterigmata and Small Conidia

Aspergillus niger van Tieghem, in Ann Sci Nat Bot s 5, t 8 p 240
1867

Synonym *Sterigmatocystis nigra* van Tieghem in Bul Soc Bot France 24 102-103 1877 See also Thom and Currie, Jour Agr Res 7 1-16 1916 and Thom and Church The Aspergilli p 167 1926

Characterization Colonies rapidly growing with abundant submerged mycelium, colorless, or in some strains with more or less yellow color in the hyphae and in the substratum, with aerial hyphae usually scantily produced, but abundant in age in certain strains. Conidial heads fuscous blackish brown, purple brown in every shade to carbonaceous black (Pl VI B), varying in intensity with the quantity of coloring matter produced, typically globose or radiate (fig 63 C) commonly up to 300-500, or occasionally 1000μ in diameter with periphery variously splitting into radiating columns of conidia small heads more or less columnar and consisting of a few conidial chains often borne on trailing hyphae or short conidiophores near the substratum. Conidiophores mostly rising directly from the substratum uncolored or yellow to brown near the vesicle only smooth with walls thick frequently uneven on the inner surface and splitting lengthwise into strips when broken (fig 64 B and C) septate or with occasional thin septa varying greatly in length and diameter in different strains and in colonies on different media or even in sections of the same colony, thus ranging from strains with conidiophores 200 to 400μ by 7 to 10μ to forms with conidiophores several millimeters long and 20μ or more in diameter. Vesicles globose or subglobose thick walled com-

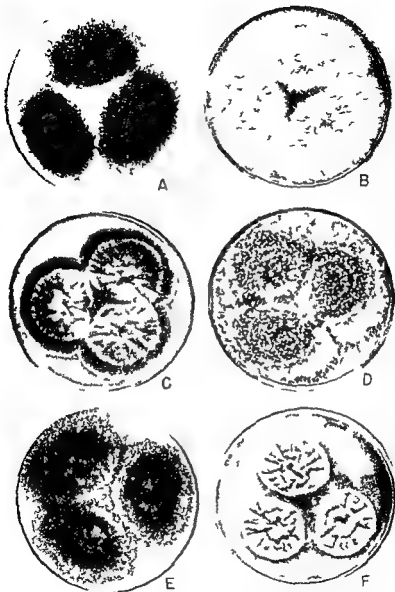


FIG 11 *Aspergillus niger* group cultures growing upon Czapek's solution agar at room temperature 10 days. A *A. niger* NRRL No. 334 typical strain. B *A. niger* mut. *schiemanni* characterized by colonies light brown in color. C *A. foetidus* NRRL No. 341 characterized by a yellowish vegetative mycelium and a strong actinomycetes like odor. D *A. niger* NRRL No. 346 characterized by the production of abundant sclerotia. E *A. phoenicis* NRRL No. 19.6 characterized by long uncrowded conidiophores. F *A. violaceo-fuscus* NRRL No. 360 characterized by compact close textured colonies and small heads with uniseriate sterigmata.

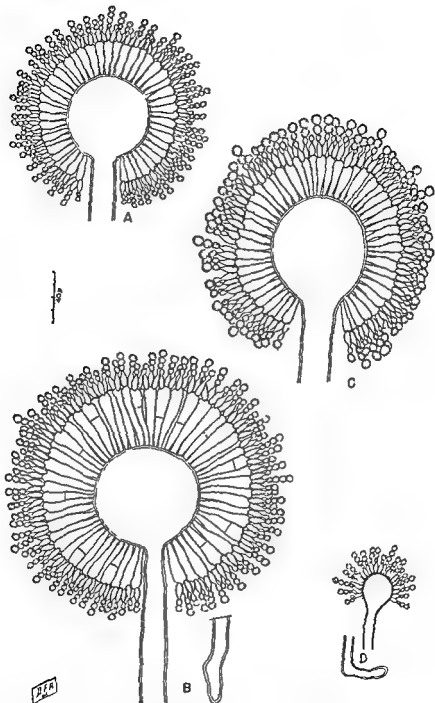


FIG 62 Conidial structures in the *Aspergillus niger* group $\times 500$ 1 *A. niger* NRRL No 346 typical head showing globose vesicle and primary sterigmata about twice the length of secondaries 2 *A. phoenicis* NRRL No 1956 characterized by long and occasionally septate primary sterigmata 3 *A. carbonarius* NRRL No 369 characterized by large primary sterigmata and large conidia 4 *A. violaceofuscus* NRRL No 360 characterized by small heads and sterigmata in a single series

monly 20 to 50 μ occasionally up to 100 μ in diameter (fig 62 A) colorless or more commonly more or less intensely yellow brown Sterigmata in one series in young colonies and in small heads but typically in two series colorless at times usually more or less intensely brown even carbonaceous primary sterigmata closely packed covering the vesicle varying greatly in size in the same colony but usually 20 to 30 μ in length by 6 to 8 μ in diameter at the outer end secondary sterigmata more uniform ranging usually from 6 to 10 μ by 2 to 3 μ (fig 62 A) both series often more or less brown to almost black Conidia globose when ripe with walls at first smooth with diffused brown or fuscous color (Ridgway Pl XLVI) then rough or spinulose from coloring substance deposited as tubercles bars or loops between the outer primary wall and the inner or secondary wall mostly 2.5 to 4 μ occasionally up to 5 μ in diameter

Sclerotia globose superficial regularly produced by certain strains (fig 61 D) sporadically by some and not found in many others

A. niger approximating the description of van Tieghem furnishes the most common morphological entity among the black *Aspergilli*. A few of the substrata and locations found in our record include chronic irritants in the human ear pin point colonies in the human lung spoiling raw sugar rancid butter and other fats floating and submerged mycelium in many chemical solutions It is abundant in soil cultures from every part of the world and apparently especially so in the tropics Molds under this name have been used in literally hundreds of biochemical investigations

The introduction of a complete description from culture for each member of the series typically represented by van Tieghem's *A. niger* but which vary from it in detailed measurements calls for repetition of many common characters In place of such descriptions the names and citations of the forms selected either as unique or as representing sections of the series often encountered are presented with the more important differences which furnish the bases of separation

Aspergillus foetidus n. sp.

Synonym *A. aureus* Nakazawa in Inst. Gov't Res. Formosa Rept. Vol. 1 1907

Not *A. aureus* Berkeley in English Flora Vol. 5 p. 346 1836

Not *S. aurca* Greco in Origine des Tumeurs et Mycoses Argentines Buenos Aires pp. 671-694 fig. 418-428 1916

Colonies upon Czapek's solution agar rather slow growing producing a floccose basal mat of mycelium with abundant but uncrowded black heads above a mass of pale orange mycelium which is deeply orange in reverse Heads up to 225 μ in diameter are borne on conidiophores about 500 μ

long, vesicles commonly 20 to 30 μ in diameter, occasionally much larger. Primary and secondary sterigmata are both 7 to 10 μ by 2 to 4 μ ; conidia are globose spinulose up to 4 or 4.5 μ .

Colonies have a penetrating actinomyces like odor (also like *Penicillium bifforme* noted in Thom, *The Penicillia*, p. 320, 1929) unlike any other species of *Aspergillus*. Numerous experiments over several years failed to justify the belief that the culture was contaminated with some actinomycete. The species is known only from Nakazawa's isolates which have maintained the odor and orange color for many years.

In the Awamori fermentation *A. foetidus* (*A. aureus* Nakazawa) was β , the unfavorable organism which gave a yellow color to the "Koji" used. Nakazawa Sumo and Watanabe (*Jour Agr Chem Soc, Japan*, No 144 pp 931-974, illustr., 1936) listed five varieties of *A. aureus* as follows: var *minor*, var *murinus*, var *acidus*, var *pallidus* and var *brevis*.

Aspergillus awamori Nakazawa in *Inst of Gov't Res Formosa Rept*
Vol 1 1907 and Vol 2 1912

The measurements given are only slightly different from those of *A. foetidus* (= *A. aureus*). The unique odor and the yellow color in the mycelium and substratum were lacking. Colonies blackish brown (deep chocolate) when heads were fully developed, conidiophores 1 to 2.5 mm by 9 to 15 μ , vesicles globose 30 to 45 μ in diameter, primary sterigmata 9 to 12 μ by 3.5 to 5.5 μ and secondary 4.5 to 8 μ by 1.5 to 3.5 μ ; conidia globose or somewhat elliptical 3 to 5 μ in long axis, fairly spinulose.

Nakazawa Sumo and Watanabe (*Jour Agr Chem Soc Japan* No 144 pp 931-974 illustr. 1936) studying the fermentation industries of Formosa found two general types of *Aspergilli* in the Awamori fermentation, α and β . Type α was *A. awamori* for which they described the following varieties: var *minimus*, var *piceus*, var *ferrugineus*, var *fuscus*, var *fumeus*. This "awamori" series produced the more desirable type of product: citric acid was present as well as alcohol due to the yeast used in the inoculum.

Aspergillus niger var *fermentarius* Nakazawa Sumo and Watanabe in *Jour Agr Chem Soc Japan* 10(2) 1934 pp 171-172 summarized on p. 181. Reported as a variety with conidiophores 1037 to 2438 μ by 111 to 160 μ ; vesicles 2.6 to 73.6 μ ; primary sterigmata 12.7 to 16.1 μ by 3.3 to 7.2 μ ; secondary 8 to 8 μ by 3.3 to 4.6 μ ; and conidia globose 2.3 to 4.6 μ in diameter. This form obviously belongs close to *A. awamori*.

Aspergillus miyakoensis Nakazawa Sumo and Watanabe in *Agr Chem Soc Japan Jour* 12(9) 963-4 fig. on 973, 1936

The colonies figured by the authors show a cottony mycelium with long stalked heads in a broad zone near the margin. The measurements

differ from *A. foetidus* as follows primary sterigmata are reported to be 12 to 20 μ by 4-4 to 9 μ in contrast to secondaries 7 to 10 μ by 2.5 to 5 μ whereas



FIG. 63. Conidia *Aspergillus niger* group. A. Single long chain of conidia $\times 1000$ (Photograph by Edward Yussl). B. Conidia of a typical strain of *A. niger* NRRL No. 344 $\times 700$. C. Conidia of the citric and gluconic acid producing strain NRRL No. 67 $\times 100$. The large size and coarsely roughened walls are characteristic of the conidia of the latter strain.

in *A. foetidus* both series measure 7 to 10 μ by 2 to 4 μ ; conidia are globose 3.7 to 5.6 μ in diameter.

The species repeats the colony appearance of certain mutants produced by means of chemical stimulants by Thom and Steinberg (1939) from the latter a standard strain of *A. niger*.

A. hennebergi Blochwitz, in Ann Mycol 33 238-9 1935

Colonies described as showing the colors and general aspect of *A. tamaris* or *A. wentii* but with conidiophores browned as in the upper part of the conidiophores of the *A. niger* group and with red sclerotia relationship doubtful. See also the *A. wentii* group

Aspergillus niger van Tieghem, in Ann Sci Nat Bot s 5 t 8 p 240 1867

Van Tieghem's strain is not fully verifiable among organisms now maintained in culture, although Biourge (personal communication) believed he had it. We believe the name can be most appropriately used to cover

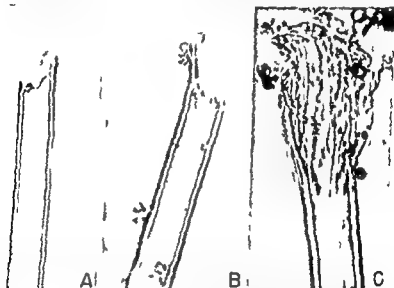


FIG 64 Broken conidiophores of *Aspergillus niger* NRRL No 376 $\times 700$. A Conidiophore showing a clean break suggesting a glass tube. B Broken conidiophore showing fibrous wall structure. C Portion of conidiophore crushed and further revealing the fibrous structure of the wall.

the exceedingly abundant isolates having the approximate measurements of sterigmata and conidia noted in van Tieghem's description. The reader can assume therefore that in the opinion of the authors any possible description for the species itself would read essentially like that already given for the series on pages 216-219.

Species Characterized by Large Primary Sterigmata and Small Conidia

A. phoenicis (Corda) Thom in The Aspergilli p 175 1926. In describing the black *Aspergillus* found upon dates Patouillard and Delacroix (Bul Soc Myc France 7 118-120 1891) compared their material to specimens

in the museum labeled *Ustilago phoenicis* and attributed to Corda, thus establishing the identity of the organism of Corda which was not recognizable from any previous references. *A. ustilago* Beck (1892) is described with the same measurements. The measurements of sterigmata place *A. phoenicis* in the section of the group with primary sterigmata 50 to 60 μ in length; it was first described as *Ustilago phoenicis* by Corda (Icones Fung. IV, p. 9, pl. 3, fig. 26, 1840) and transferred by Patouillard and Delacroix as noted above to *S. phoenicis* (Corda) Patouill. and Delacr. If we accept the use of *phoenicis* attributed to Corda as correct, this species becomes the type of the section of the black *Aspergillus* with primary sterigmata of intermediate length and conidia not over 4 μ in diameter (fig. 61 E and 62 B). This was cited by Thom and Currie as *A. phoenicis* (Corda) Pat. and Delacr. and continued recognition of the species to cover a group of strains occasionally encountered appears warranted.

Aspergillus pulverulentus (McAlpine) Thom in Jour. Agr. Res. 7: 10-11, 1916.

Synonym *S. pulverulenta* McAlpine in Agr. Gaz. N. S. Wales (1896) 7: 302, 1897. See also Thom and Church, The *Aspergilli*, p. 179, 1926.

McAlpine's data include: White to dirty yellow mycelium; heads 155 to 340 μ in diameter; radiate with chains mostly separate; conidiophores erect, stiff, up to 7 mm. by 20 μ , with walls up to 5 μ thick; vesicles 70 to 170 μ in diameter, globose or nearly so; primary sterigmata up to 144 μ long by 8 μ ; secondary 14 to 18 μ long; conidia globose, rough, about 4 μ in diameter. Colonies with these general characters have been studied in culture at least twice and maintained for long periods: one came from Spain, the other from Texas. *A. strychni* Lindau (Hedwigia Bd. 43, Rept. 5, p. 306-7, 1904) is one of this series.

Light Colored Forms

Aspergillus niger mut. *cinnamomeus* (Schuermann) n. comb.

Synonym *A. cinnamomeus* Schuerm. in Ztschr. Induktive Abstamm. u. Vererbungslehre Bd. 8, Heft 112, pp. 1-35, 16, fig. 2, pl. (1 col.), 1912. See also Thom and Church, The *Aspergilli*, p. 164, 1926.

Colonies upon Czapek's solution agar at room temperature rapidly growing and spreading, producing an aërial growth of conidiophores and heads reaching a pale cinnamon upon maturity. Reverse only slightly

colored in the same shade. Conidial heads not crowded globose. Conidiophores smooth, thick walled, with upper portion more or less brown, about 1.5 mm in length by 12 to 20 μ in diameter. Vesicles up to 40 to 50 μ in diameter, crushing readily. Sterigmata in two series primary about 15 to 20 μ by 3 to 5 μ , sometimes larger, secondary about 8 by 3 to 3 μ . Conidia 3 to 4 μ , thin walled globose or subglobose smooth or nearly so almost colorless when viewed singly, pale yellowish to cinnamon in mass.

Diagnosis based upon culture NRRL No 348 (Thom No 3534b) received from Schiemann as a mutation induced by introducing potassium bichromate into the culture medium. Approximately the same mutant appeared in Steinberg and Thom's series of induced mutations (1939, 1940). Occasional cultures close to *A. cinnamonaceus* have been obtained from unknown sources in nature.

Aspergillus niger mut *Schiemannii* (Schiemann) n. comb.

Synonyms *A. Schiemanni* (Schiemann) Thom, Jour Agr Res 7 13 1916

A. fuscus Schiemann, in Ztschr Induktive Abstam u Vererbungslehre Bd 8, Heft 1/2, p 1-35, 10 fig 2 pl (1 col) 1912

Not *A. fuscus* Bonorden (Bot Ztg Jahr 19 202 1861),

Not *S. fusca* Bainier (Bul Soc Bot France 27 29, Pl 1, fig 5 1880)

Colonies upon Czapek's solution agar at room temperature rapidly growing and spreading, developing a surface growth of conidiophores and heads forming a crowded fruiting area 2 to 3 mm deep in slanted tubes, becoming a shade of brown near fawn color (Ridgway, Pl XL) reverse yellowish (fig 61 D). Conidial heads large, fairly crowded. Conidiophores coarse, 2.5 mm or more long by 15 to 25 μ wide. Vesicles up to 50 to 60 μ in diameter. Sterigmata in two series, primary 15 to 40 μ by 4 to 6 μ , sometimes larger secondary 7 to 8 μ by 2 to 3 μ . Conidia thin walled smooth except sometimes a trace of markings 3.5 to 4.5 or 5 μ in diameter.

Culture NRRL No 361 (Thom No 3534C) was received from Schiemann. Culture NRRL No 362 was received from Bourge under the same name but shows a much deeper brown color (near Natal brown Ridgway Pl XL).

The mutant *A. niger* mut *Schiemannii* is distinguished from the parent type of *A. niger* by the color and smoothness of its spores. The name, *A. fuscus* Schiemann is invalidated by the prior usage of the names *A. fuscus* by Bonorden and *S. fusca* by Bainier.

Mutations of approximately this same type have been secured from cultures of strain NRRL No 67 (Pl VI C) by ultra violet irradiation.

(Raper Coghill and Hollaender), from cultures of other black *Aspergillus* through Cathode ray irradiation (Whelden 1939) by cultivation in the presence of various chemicals (Steinberg and Thom 1939 1940) and finally by the appearance in plate culture under normal conditions of a light-spored sector in an apparently typical culture of *Aspergillus niger*. See discussion Chapter VI. Two isolates labeled *A. awamori* Nakazawa which resemble this mutant in color have been received from Japan one from Hanzawa the other from Nakazawa. Both produce primary sterigmata less than 20μ in length but otherwise are close to Schiemann's mutant.

Probable Synonyms

Many species have been described by investigators working at different periods and at widely separated stations which obviously belong within the *Aspergillus niger* series as it is here considered. Some of these will be briefly noted since they were reported to present unique cultural or morphological features or since they account for interesting or important biochemical reactions.

A. giganteus Matile (Ann Soc Belge Med Trop 6 36 1936) was described as a new species with conidiophores 2 to 6 mm long conidia 4 to 5μ in diameter rough black brown but without other marks of separation. It would seem to have been a rather extreme variant in the two characters.

A. atropurpureus Blochwitz (Ann Mycol 32(1/3) 86 1934) Not *A. atropurpureus* Zimmermann (Centralb f Bakt etc 2 Abt 8 No 5/7 p 218 1907) Blochwitz proposed to use the name *A. atropurpureus* for the purple brown members of the *A. niger* group as he obtained them from the tropics. He gave conidial measurements as 2.5 to 3 μ in diameter and added that chemical differences in the coloring substances warranted separation from the black or blacker forms.

A. fuscum (Reich) Hennings in Hedwigia 34 p 86 1895 and Reichardt in Verhandl K K Zool Bot Gesell Wien 27 335 1867. Regarded as *A. niger* by Wehmer in Centralb f Bakt etc 2 Abt 18 No 13/15 p 394-395 1907. See also Thom and Church The Aspergilli p 174 1926.

Slight differences in morphology between this and *A. niger* v Tieg are reported by Hennings but disregarded by Wehmer. Culture NRRL No 364 (Thom No 112) received in 1908 from Westerdijk under this name was reported by Thom and Currie (1916) as the most rapid producer of oxalic acid of all strains of *A. niger* tested. There is no morphological basis for separating this strain from *A. niger* and it is distinct in culture only by the tendency after many years in laboratory culture to produce rather floccose colonies.

A. datatos Saito in Centralb f Bakt etc 2 Abt 18 No 1/3 p 34 1907. Saito's organism as described is close to *A. niger* colonies reach brownish black through yellow shades conidiophores 2 to 4 mm by 12 to 20 μ smooth thick walled brown in upper portion vesicles 35 to 4μ globose primary sterigmata 24 to 40 μ long by 3μ at apex secondary 10 by 3 μ conidia globose brown finely roughened 4 to 5μ in diameter. A culture under this name from Formosa (NRRL No 383) presented the

comparatively large conidia described by Saito but possessed no other marks which would assist in identification

A. luteo niger (Lutz) Thom and Church in The Aspergilli p 166 1926 Syn *S. luteo nigra* Lutz in Bull Soc Bot France 53 48-57 1907 Thom and Church (1926) found one or more black Aspergilli in which the conidia appeared smooth in ordinary laboratory slide mounts in which they were treated with alcohol followed by lacto phenol or other mounting fluids Fragments floating in the mounting medium led to the test of such conidia in dry mounts in oil and in pure glycerine Under these conditions the conidia showed the typical marks of the *A. niger* series Since smooth conidia were the sole definite contrast between *A. luteo niger* Lutz and *A. niger* v Tiegh the strains studied were accepted as Lutz organism Since that time, it has been shown that many black Aspergilli ranging widely among the variant forms encountered pass through a physiological stage in which the outer wall and color bars when subjected to alcohol or other fluid causing active osmotic currents break in pieces and float away leaving the conidia smooth and colorless or only partly shaded toward the dark color of the group It thus appears that *A. luteo niger* may be dropped as failing to designate any definite group of strains and as failing to present any character of diagnostic importance

ASPERGILLUS CARDOARIUS SERIES

Species Characterized by Conidia in Excess of 5.0μ—Sterigmata in Two Series

Aspergillus atropurpureus Zimmermann, in Centralb f Bakt, etc, 2 Abt, 8, No 5/7, p 218 1902

Conidiophores hyaline or somewhat brownish in age up to 800 by 16μ to 20μ, vesicles 60 to 80μ in diameter, hyaline to brown sterigmata primary 16 by 6μ secondary 3 to 4μ by 1.5 to 2.0μ conidia globose rough with prominent warts, purplish black, 6 to 10μ in diameter Isolated from *Coffea liberica*, in Java Culture not studied by us The species is possibly valid and is presented as representative of occasional forms characterized by small sterigmata and large purple black spores

Aspergillus fumaricus Wehmer in Ber Deut Chem Gesell 51 No 14 1663-1668 figs 1-6 1918

This species was named but not fully described by Wehmer and was not distributed by him Its biochemical activity as a producer of fumaric acid is covered in the paper cited above together with the admission that it belonged to the *A. niger* group Culture No 4668 2 (C Thom) received from Neuberg under this name presents a variant strain of the group which may be described as follows Colonies producing a mass of yellow mycelium, conidiophores scantily and tardily produced up to 1.2 or 3 mm long, by 20 to 22μ in diameter smooth, bearing large radiate yellow brown heads Vesicles up to 60 or even 100μ in diameter with walls thin easily crushed Sterigmata in two series primary sterigmata up to 15μ by 4 to

5 μ secondary sterigmata rather coarse some growing out into aborted hyphae. Conidia commonly 5 μ occasionally 7 to 8 μ in diameter with lengthwise color bars as in *A. niger* but paler in color.

Culture No 4668 \bar{E} (Thom) is accepted as correctly named. By description, *A. fumarius* Webber differs little from *A. atropurpureus* Zimm except for the production of yellow brown rather than purple black conidial heads. Differences in color definition and discrimination by different workers tend to leave both species in doubt.

Aspergillus fonscaeus n. sp.

Synonym *S. fusca* Bainier in Bul. Soc. Bot. France 27: 29 Pl. 1 fig. 5 1880 (Bainier's material grown upon moist bread in Toulouse in 1880 was preserved in Roumeguère's Fungi Gallici Exsiccati No. 995).

Bainier described *S. fusca* in terms closely parallel to van Tieghem's *A. niger*, except that the conidia were about double that species in size. Bainier reported these as rarely exceeding 9.4 μ in diameter, while our examination of the exsiccati showed them to be mostly 5 to 8.5 μ in diameter and marked \equiv in *A. niger* and *A. carbonarius*. Da Fonseca in Rio de Janeiro contributed a culture (Thom No. 4707-878, NRRL No. 67 and strain No. 67 of Herrick May, and associates) possessing approximately these spore measurements which has retained its characteristic features for more than 20 years and which has proved industrially useful. We believe therefore that recognition of a species with sterigmata of intermediate length and conidia about double the dimensions of *A. niger* van Tieghem is warranted. The name *A. fonscaeus* is proposed since the binomial *A. fuscus* had already been used for an *Aspergillus* by Bonorden in 1861 (Bot. Ztg. Jahrg. 19 No. 29 p. 202) and has been used at least twice subsequent to this. The species would then include strains characterized by large subglobose coarsely roughened conidia ranging from 5.5 or 6.0 μ to 8.5 or 9.0 μ (fig. 63 C) including the strain 67 used by Herrick May Wells Moyer et al. of the Industrial Farm Products Research Division U. S. Department of Agriculture Arlington Farm Virginia for the production of citric and gluconic acids (see literature citations pp. 290-295).

The following description is based primarily upon strain NRRL No. 67.

Colonies upon Czapek's solution agar growing rapidly at temperatures from 24 to 30 C attaining a diameter of 7 to 8 cm in eight to ten days consisting of a basal vegetative mycelium that is largely submerged and colorless and abundant conidial structures commonly arranged in more or less conspicuous concentric zones heads carbon black or brownish black imparting to the colony a like coloration reverse colorless in young colonies.

commonly darkening in age and often becoming almost black after 2 to 3 weeks. Conidial heads large, globose, radiate or with chains of conidia massed in an indefinite number of loose divergent columns, commonly 300 to 500 μ but often attaining a diameter up to 1 mm. Conidiophores varying in length from 1.5 to 3.5 mm but averaging about 2.0 to 3.0 mm, mostly 20 to 30 μ in diameter with walls 2.0 to 3.0 μ thick, smooth, often colored in dark shades in the region beneath the vesicle. Vesicles globose, fertile over the entire surface, commonly 50 to 75 μ in diameter (fig. 62 C), usually in brown shades, often quite dark. Sterigmata in two series, usually brown, often dark. Primary sterigmata variable in different heads and in different cultures, ranging from 15 to 20 μ by 11 to 8 μ in some to 35 to 45 μ by 10 to 13 μ in others. Secondary sterigmata ranging from 8 to 14 μ by 5 to 6.5 μ but averaging about 11 to 10 μ by 5 to 6 μ . Conidia large (fig. 63 C) globose, conspicuously roughened with prominent color bars, ranging from 5.5 to 8.5 μ .

Since strain "67" appears in the industrial fermentation literature as *Aspergillus niger* and has been consistently distributed under this name over a period of several years, it is not our purpose here to challenge this designation, for this binomial is often used in a very general sense to cover any black member of this group. We do wish to emphasize however that this strain does not represent the common type of black *Aspergillus* usually isolated in routine examination of soil and moldy materials in general. The fact that it possesses large spores is of the greatest value in checking its purity and further commends it for use in industrial operations. This strain was originally received from Rio de Janeiro and recently Dr. Dorival M. Cardoso of Sao Paulo, Brazil, has reported (personal communication) that he has repeatedly isolated large spored forms apparently closely related to it. It would, therefore, appear likely that this large spored form represents a type of organism much more abundant in South America than in this country.

Aspergillus pulchellus (Spez.) Thom and Church in *The Aspergilli* p. 181
1926

Synonym *Aspergillopsis pulchellus* Spegazzini in *Myc. Arg.* 1 in *Ann. Mus. Nac. Buenos Aires* Ser. 3 t. 13: 436 1911

This species was described with colonies intensely black, conidiophores 1 to 2 mm by 18 to 20 μ with walls darkened, vesicles 50 to 60 μ in diameter, primary sterigmata 10 by 30 μ and conidia 8 to 10 μ in diameter and rough. The species appears to differ from van Tieghem's *A. niger* principally in its very large conidia, and it is extremely doubtful if it could be separated from *A. fonscaeus* as described above.

A. dipus of Ferdinandsen and Wing (Bot. Tids. 30: 220 fig. 6 1910) represents another organism undoubtedly close to the forms under consideration. The presence of conspicuous foot cells upon which character the species was based is common to all members of the group, hence is not a valid basis for separation.

Aspergillus carbonarius (Baumer) Thom in Jour Agr Res 7 12 1916
 Synonym *S carbonaria* Baumer in Bul Soc Bot France 27 27-28 1880

Colonies grown upon Czapek's solution agar show vegetative mycelium white or with some yellow in submerged areas broadly spreading more or less zonate sclerotia produced upon the surface of the substratum in old cultures fruiting areas carbon black Conidiophores colorless below yellow to yellow brown toward the apex 4 to 6 mm or more in length and up to 25 μ in diameter with walls smooth sometimes as much as 4 μ in thickness Heads globose varying in diameter up to 500 μ Vesicles up to 90 μ in diameter fertile over the entire surface commonly with contents yellow brown to black and in old heads forming with the primary sterigmata a hard brittle carbonaceous mass Sterigmata in two series primary sometimes one septate from 20 to 40 μ long in young or small heads and up to 120 μ long in large heads by 5 to 13 μ in diameter at the apex secondary 8 to 14 μ by 3 to 6 μ Conidia at first smooth becoming rough when ripe 5.5 to 10.5 μ in diameter Colonies grow well upon all culture media used with temperature optimum below 37 C A culture from Dr A F Blakeslee (ARRL No 369 Thom No 4030 I) reproduces in detail the morphology recorded by Baumer *A carbonarius* has also been received in culture from the Gold Coast of Africa

The same morphology was also found in one of Dr Farlow's specimens *S acinutae* Caballero (Bul R Soc Esp Hist Nat 28 429 1928)
 This was described as it appeared upon rotting grapes as follows vesicles 75 to 100 μ by 73 to 98 μ primary sterigmata 59 to 80 μ by 13 to 22 μ and secondary 15 to 20 μ by 5 to 7 μ conidia globose rough 6 to 10 μ Blochwitz in Die Aspergillaceen (Ann Mycol 27(3/4) 204 222 and 232 1929)
 with part of the type specimen before him declares it to be *A carbonarius* apparently without cultivating it Mowseray (LaCellule 43 222 1934)
 places Caballero's organism in *Aspergillus pulchellus*
 We cannot agree with Blochwitz (idem p 221 222 fig 12) in describing the vesicle in *A carbonarius* as subglobose and figuring the head as hemispherical Baumer's original figures show the vesicle fertile to the very base This is characteristic of the strains observed from various sources

ASPERGILLUS LUCHUENSIS SERIES

Species Normally Showing One Series of Sterigmata Occasionally Two
Aspergillus luchuensis Inui in Jour Col Sci Imp Univ Tokyo 15 469
 Pl 22 fig 1-8 1901 See also Usami in Centralb Bakt
 etc 2 Abt 43 p 200 1915 The Aspergilli
 p 171 Pl IV 1926

Colonies upon Czapek's solution agar spreading rapidly producing abundant conidiophores and conidial heads which give a purple black color

to the whole colony, reverse in pale yellow shades. Conidial heads globose, up to 250 to 300 μ in diameter, splitting in age into short columns of spores. Conidiophores up to 1500 μ by 10 μ smooth yellow toward the vesicle. Vesicles yellow, up to 40 μ in diameter fertile over the entire surface. Sterigmata mostly in one series 6 μ or a little larger by 3 μ , branched sterigmata occasionally appear. Conidia globose, 3.5 to 4 μ roughened with spines rather than bars of coloring substances.

Representatives of the species have been received from Japan (NRRL No 306, Thom No 42913) from West Africa from Bermuda, and from various points in the United States. While not as abundant as forms with double sterigmata, isolates with this kind of head are not uncommon.

Inui also described *A. permiosus* (Jour Coll Sci Tokyo 15 p 473 T XXI, figs 9-12 1901) with color data closer to *A. ventus* than *A. luchuensis*. Its morphology however seems to belong here. It has not been rediscovered and discussed adequately in relation to either group. Culture No 4707 707 (Thom), received from da Fonseca in Brazil, possibly represents this species; colonies in center at least transiently greenish but without true green color; conidiophores not crowded sinuous apparently smooth when observed with low magnifications but with traces of pitting evident when examined with an oil immersion objective up to 1000 μ by 10 to 15 μ primary sterigmata 10 to 16 μ by 3 to 4 μ secondary up to 8 μ by 2 to 3 μ , conidia 4 to 5 μ in diameter with yellow markings in the form of loops and bars.

A. luchuensis var *rubeolus* Shih (Lingnan Sci Jour 15(3) 374 1933) differs from the species by becoming chocolate brown rather than black. This would suggest careful comparison with *A. japonicus* Saito.

Aspergillus japonicus Saito in Bot Mag (Tokyo) 20 61 5 figs 1906

Colonies upon Czapek's solution agar growing rapidly and spreading evenly characterized by its purple brown heads and the presence of few to many light brown sclerotia, reverse colorless or nearly so. Conidiophores 500 to 1000 μ by 12 to 15 μ figured as showing concretions on the surface with walls more or less brown. Vesicles globose fertile over the whole surface with walls brown and marked by the bases of sterigmata. Sterigmata in one series 7 to 9 μ by 5 to 6 μ commonly falling away in mounts from old cultures. Conidia globose echinulate 4 to 5 μ in diameter. Sclerotia scattered throughout the colony 650 to 1000 μ in diameter white to pale yellow in color and often overgrown by mycelium and conidiophores.

Type material has not been seen. The diagnosis is based upon two strains contributed by Dr A F Blakeslee (NRRL No 308 Thom No 4030 3 and NRRL No 359 Thom No 4030 5) which come fairly close to

Saito's description. A purple brown strain (Thom No 5362 7) collected by Manns in Honduras showed lightly darker colors and slightly different measurements. A strain received from Raistrick as coming from Blochwitz through the Centralbureau in Bonn labeled *A. atropurpureus* Zimmermann resembled the two Blakeslee cultures quite closely. It certainly did not comply with Zimmermann's description (see p 226). *A. luchuensis* var *rubcolis* Shih is probably closely related if not identical with *A. japonicus* Saito.

Aspergillus violaceo fuscus Gasperini in Atti Soc Toscana Sci Nat Pisa
Mem 8 fasc 2 p 326 1887

Colonies upon Czapek's solution agar comparatively slow growing (fig 61 F) purplish brown with a faint violet shade passing to purple drab in age reverse colorless to dark purplish. Conidial heads purplish brown, globose not crowded 100 to 150 μ in diameter. Conidiophores mostly less than 1 mm in length but sometimes reaching 2 mm 12 to 18 μ in diameter. Veleles globose varying up to 60 μ in diameter. Sterigmata generally in one series (fig 62 D) 5 to 8 μ by 3 μ occasionally in two series with secondaries 2 to 4 μ long. Conidia elliptical 3.5 to 5 μ by 5 to 6.5 μ at first hyaline becoming violaceous somewhat roughened.

By description this is a variant member of the great group of black *A. pergilli* characterized by short sterigmata and elliptical conidia.

Gasperini's material has not been seen but three cultures have been studied which are believed to differ from his species only in having somewhat smaller conidia. The first of these was received in 1914 from Puerto Rico (NRRL No 360 Thom No 3522 30) while the others were subsequently obtained from Jamaica and from Professor Raistrick in England.

In cultivating black *A. pergilli* an occasional strain produces heads at first showing a single series of sterigmata then as the colony becomes older heads with both primary and secondary sterigmata dominate the culture. Upon careful examination many strains show both large heads with sterigmata in two series and on shorter conidiophores mixed among the larger ones small heads with simple sterigmata only or with both types mixed. Colonies of this kind probably accounted for *A. nanus* Montagne *A. subfuscus* Johan Olsen (Sopp) and are known to account for *A. pyri* English.

Aspergillus nanus Montagne in Syllog Generum Specierumque Cryptogamarum
■ 300 No 112 Paris 1856 Sacc Syll 4 71 1886. Species reported as a member of the black group with a single series of sterigmata about 15 μ in length and spores 3 μ in diameter. This may have represented young fruiting structures of a typical *A. niger*.

Aspergillus subfuscus Johan Olsen in Meddelelser fra Naturh forening i Kris

tiana 1885. Described as showing smooth globose spores $3\frac{1}{2}$ to 3.5μ and a single series of sterigmata in culture up to 20μ in length but with some secondary sterigmata seen in the original material. The separation of this from *A. niger* appears questionable.

Aspergillus pyri English n n in Doctoral Thesis State College of Washington Pullman Wash pp 76-78 1940 cited in abstract. A form described as showing a single series of sterigmata 14.4 to 19.2μ by 3.6μ and spinulose conidia 3.6 to 4.8μ in diameter. In personal correspondence of June 1943 English stated that as a result of continued study of this strain and the finding of double sterigmata he questioned the desirability of maintaining the species designation.

SYNOPSIS OF SPECIES PROPOSED BY MOSSERAY

Biourge, who was a discriminating collector, accumulated 63 strains of "black" (or related) *Aspergilli* for each of which he could see sufficient individuality to warrant preservation. Mosseray, working in Biourge's laboratory, studied these strains, attempted to establish species lines among them, and proposed new specific names for all forms which he believed undescribed. With the whole 63 strains in parallel culture, all bearing the names applied by Mosseray, Biourge and Simonart were inclined to withdraw part of Mosseray's new species names a position with which the writers heartily agree. Nevertheless to present one concept of the range of variation confronted by the student of this group Mosseray's synopsis* has been translated with minor emendations, and is herewith presented.

Mosseray's Synopsis

A. Conidia 6 to 10μ in diameter rough vesicles subglobose primary sterigmata often 100μ or more in length colonies jet black

a. Sporulation more or less dense heads large reverse fumose or very dark olive with mycelium more or less wrinkled

Conidiophores $2-4$ or even 6 mm long sclerotia present in 'natural' media

A. carbonarius (Bainier) Thom and Currie

Conidiophores 1 to 2 mm long sclerotia not reported *A. pulchellus* (Speg.)

Thom and Church Syn *S. acinus* var. *Caballero*

b. Sporulation less dense heads small also with very small heads with single

* Translated and emended from Mosseray Raoul Les *Aspergillus* de la section *niger* Thom and Church in La Cellule XLIII 271-273 1934. All data are based upon colonies grown in slanted test tubes using Biourge's formula of neutral Raulin agar of the following composition:

Water (distilled)	1000 cc	$(\text{NH}_4)_2\text{HPO}_4$	0.400 g
Sucrose	50 g	$(\text{NH}_4)_2\text{SO}_4$	0.700 g
Tartaric acid	0.40 g	FeSO_4 , cryst	0.050 g
MgCO_3	0.250 g	ZnSO_4	0.050 g
NH_4NO_3	2.500 g	Agar	20.0 g
K_2CO_3	0.400 g		

Sterilized at 120°C for 20 minutes

sterigmata and on very short conidiophores among them mycelium gray yellow reverse dark reddish brown and mycelium much wrinkled

A. pseudo-carbonarius (Bainier n n) Mosseray

- II Conidia 2.5-4 even to 6 μ in diameter mostly more or less rough and more or less colored but some smooth or nearly so vesicles normally globose primary sterigmata mostly 20 μ long or longer sometimes up to 100 μ

- a Colonies deep purple brown or clear purple brown

- I Conidiophores short (up to 2 mm on an average)

- a Wrinkles in reverse shallow transverse (in tubes)

Heads small conidiophores short (up to 1 mm) 8 to 15 μ in diameter primary sterigmata 5 to 20 μ reverse cream colored

A. microcephalus Mosseray

Heads medium conidiophores up to 2.5 mm primary sterigmata up to 45 μ reverse dark reddish brown often spotted and becoming very dark.

A. phoenicia (Corda) Thom

Syn *A. longobardica* (Bainier n n) Mosseray

Syn *A. bainieri* Mosseray 1934

- g Wrinkles in reverse of mycelium fairly numerous occasionally anastomosing

Appearance mealy deep purple brown conidiophores very short up to 0.5 mm and 7 to 13 μ in diameter primary sterigmata 4 to 12 μ reverse deep olive brown

A. pseudo-citrinus Mosseray

Appearance sub granular brown purple at times clear brown to umber conidiophores up to 1.5 mm by 7 to 90 μ conidia smooth or nearly so growth rapid Reverse slightly colored

Reverse cream to pale brown primary sterigmata 8 to 20 μ

A. fuliginosus Peck

Syn *S. fuliginosa* Bainier

Syn *A. praecox* Mosseray 1934a

Reverse very deep brown primary sterigmata up to 50 μ sclerotia occasional

A. sclerotifer Mosseray

Reverse uncolored to citron yellow primary sterigmata 8 to 20 μ

A. citrinus niger Mosseray

- 7 Wrinkles sharp and numerous (reticulated) Reverse becoming rapidly dark almost black

Aspect mealy color purple brown conidiophores up to 1 mm drops not colored primary sterigmata 11 to 30 μ conidia smooth or nearly so

A. densus Mosseray

Aspect mealy to granulate deep purple brown drops numerous bronze primary sterigmata 12 to 30 μ

A. rutilans Mosseray

Aspect subgranular brown purple drops numerous black some of them very large primary sterigmata 1" to 30 μ

A. guttifer Mosseray

Reverse not so dark sometimes olive or colorless

Reverse deep olive often spotted primary sterigmata 15 to 30 μ

A. Buntingii Mosseray

Reverse not colored commonly showing areas sterile or free from spores mycelium slightly yellow at first primary sterigmata 20 to 50 μ

A. variegatus Mosseray

II Conidiophores up to 3 mm rarely 4 mm long

α Sporulation normal

Reverse uncolored or slightly olive drops few or none primary sterigmata 20 to 40μ mycelium often yellow at first

A. niger Van Tiegham

Reverse orange brown or purple brown drops large black primary sterigmata 8 to 25μ mycelium colorless or rarely yellow

A. Bourgei Mosseray

Reverse pale reddish brown drops very few heads very small primary sterigmata 15 to 30μ mycelium colorless or sometimes rose sporulation very slow

A. Churchii Mosseray

Reverse golden yellow, drops none primary sterigmata 20 to 40μ mycelium reddish yellow at first

A. luteo niger (Lutz) Thom and Church

Reverse dark olive brown conidiophores up to 1 to 2 mm primary sterigmata very variable vesicles subglobose

A. anomalus Mosseray

Reverse cream to brown spotted slightly wrinkled with a dark band in center sporulation scattered pale conidiophores up to 4 mm long

A. tubingensis (Schöber) Mosseray

β Sporulation abnormal

Sporulation absent in patches and in the margin with a granular appearance and proliferation of mycelium throughout the conidial area

A. granulatus Mosseray

Sporulation massed at the thin end of the agar less dense toward the bottom of the tube mycelium wooly

Mycelium wooly gray white or yellowish gray carrying on the thin areas numerous simple heads (fumigatiformes) and some normal heads Reverse cream slightly wrinkled

A. telinus Mosseray

Mycelium less wooly white conidiophores 1 to 3 mm long very abundant at the thin end of the agar the remainder sterile Reverse much wrinkled cream

A. ficum (Reich) Hennings

III Conidiophores tall up to 1 cm sporulation scanty mostly toward the thin end of the agar

Heads large splitting into divergent columns conidiophores up to 1 cm by 30μ primary sterigmata up to 100μ reverse cream or slightly rose

A. elatior Mosseray

Heads small mycelium gray rose conidiophores rarely up to 1 cm more often 2 to 6 mm very numerous little fumigatiform heads on short conidiophores at the surface reverse clear rose or salmon

A. pseudo elatior Mosseray

IV Conidiophores very irregular from less than 1 mm to 3 mm with much larger heads reverse clear reddish brown mycelium yellow at first then dark brownish red sporulation delayed

A. pseudo niger Mosseray

■ Colonies not purple brown but clear brown morphology of *A. niger* conidia mostly smooth or nearly so

I Colonies clear brown or sepia conidiophores up to 1 mm reverse olive or lighter with reticulated wrinkles vesicles 20 to 50μ primary sterigmata 12 to 50μ conidia smooth

A. olivaceo fuscus Mosseray

- II Colonies umber conidiophores 1 to 3 mm reverse reticulated dark reddish brown conidia smooth or nearly so 4 *Schimmanni* Thom
Syn *A. fuscus* Schimmann
- III Colonies reddish salmon conidiophores 1 " or even 3 mm reverse slightly wrinkled uncolored or pale cream conidia smooth
A. cinnamomeus Schimmann

C Conidia 3 to 5 μ globose smooth slightly colored vesicles globose conidiophores up to 1 mm slender primary sterigmata 12 to 20 μ colonies appearing somewhat granular deep brown reverse olive passing to bronze mycelium sulphur yellow at first
A. citricus (Wehmer) Mosseray

D Conidia 3 to 5 μ globose or elliptical smooth or slightly rough colonies violaceous
a Sterigmata in two series vesicles globose colonies purplish violet or mauve reverse violet brown conidia 3 to 5 μ globose smooth uncolored

A. aurum Uesam

b Sterigmata in one series short vesicles subglobose colonies in violaceous shades to mauve

Colonies mauve (violet livide) reverse uncolored wrinkled conidia globose or obovate smooth 3 to 5 μ in long axis sporulation slow narrowly growing
A. malsaceus Mosseray

Colonies violaceous or dark violet slate reverse dark yellow or orange slightly wrinkled conidia globose and rough 3 to 4.5 μ sporulation very rapid and colonies broadly spreading with sclerotia common on rice or other natural substrata rare upon sugar media 1 *japonicus* Saito

Colonies violet brown reverse purplish brown wrinkled conidia globose rough 3 to 5 μ in long axis sporulation slow and more or less incompletely covering the surface dwarf heads abundant *A. atro violaceus* Mosseray

Colonies dark brown or carb brown with a mealy or granular appearance reverse dark brown or olive at the margins wrinkled conidia smooth globose 2.5 to 4 μ vesicles globose or pyriform primary sterigmata 3 to 7.5 μ in diameter
A. atro fuscus Mosseray

If the material available for study were limited to Biourge's 63 cultures identification to strain variety or species might be possible. When Mosseray subsequently returned to Brussels as Mycologist at the Jardin Botanique de l'Etat and was confronted by hundreds of other strains largely from the Belgian Congo many of which presented further variation he began to see the impossibility of describing them all in terms which would permit subsequent identification. Biourge at that point (personal conference) withdrew his support from many of the diagnostic features accepted in Mosseray's *mémoire* and agreed to the proposal that specific names in black *Aspergilli* could only be serviceable as bringing together aggregates of strains showing common and fairly dependable morphological characters. This was the attitude held by Thom and Church in *The Aspergilli* (1926) based upon the examination of many hundreds of black *Aspergilli* and it remains the position of the writers at this time.

Since variation is characteristic of the whole genus—not one group

alone, the subject is covered in the chapters on Morphology and on Variation

Coloration

Color has been emphasized in most of our attempts to separate these strains in culture. Linossier (1891) extracted from his strain of *A. niger* a coloring substance soluble in hot water which he called aspergilline. This substance is readily demonstrated. Blochwitz (1929, p. 219) reports this material to be soluble in " NH_3 and alkalis" and we have extracted it with hot water. Microscopic comparison of conidiophore heads, and separate conidia from numerous strains leads to the conclusion that the same substance may give a brown color to the upper one third of the conidiophore, to the vesicle and its contents, to the walls of the sterigmata, and in *A. carbonarius* so fill the whole vesicle and sterigmatic area with a brittle mass as to justify Baurier in calling the head carbonaceous. Conidia in a few strains have appeared to possess smooth, uniformly brown cell walls. As a group however the black aspergilli have globose conidia with a firm, uncolored or slightly colored inner wall, a very thin outer wall and between the two the coloring substance presumably aspergilline deposited as granules, warts or bars running lengthwise of the cells and presenting a pattern characteristic of the whole group. When pale colored variants (mutants?) such as *A. cinnamomeus* (p. 223) or darker ones such as *A. schiemanni* (p. 224) or the variously brown to deep carbon black ones like *A. carbonarius* are compared the relative quantities of coloring matter seem to account for the progressive darkening of the head colors as described in the series.

In the 'reverse' or underside of the mycelium and in the culture substratum, a range of shades from colorless to yellows to reddish brown and even very dark shades, is reported for particular species growing upon specified substrata. The chemical reactions back of these colors are not known for the black aspergilli. Blochwitz has reported extractions of color from various species of *Aspergillus* and other molds and observed that variations in these colors were readily obtained with reagents. Some of the extracted material were reported to act as indicators. It is therefore, doubtful whether the succession of colors present in such a related series of organisms represent substances differing fundamentally one from another, or merely successive steps in the transformation and reactions of the same general type of product.

Occurrence and Economic Importance

The black aspergilli are probably more common than any other representatives of the genus. They are world wide in distribution and occur

in and upon the greatest variety of substrata including grains forage products spoiled fruits and vegetables exposed cotton textiles and fabrics leather dairy products and other protein rich substrata and decaying vegetation in the field They are abundant in all soils examined, and from studies which have been made by the authors and other investigators, it would appear that they are particularly abundant in soils from tropical and sub-tropical areas

With the possible exception of the *A. flavus-oryzae* group which is of great economic importance in the Orient the black aspergilli are undoubtedly more widely used in industry than any other group of molds Since the present volume is primarily a manual designed to assist the worker in the study diagnosis and maintenance of the aspergilli that come into his hands no attempt will be made to discuss the various fermentations and other biochemical activities of the black aspergilli However these fermentations are of great importance and will be briefly noted For the reader who is interested in these fermentations or perchance is actually conducting them, a fairly complete list of references is presented for each in the Topical Bibliography (Chapter XXII) In each case it has been our aim to present sufficient references to provide the reader with a reasonably comprehensive guide to the literature of the field

Gallic Acid Raulin and his coworkers in Paris during the early 1860's identified the organism active in the production of gallic acid by the fermentation of gallnuts and other tannin bearing substances The species was first discussed as *Ascophora nigrans* Van Tieghem in 1867, named and described the organism correctly as *A. niger* Recurrent investigations have been conducted on this fermentation from that period to the present time (see Topical Bibliography p 294)

Citric Acid In 1917 Currie published his fundamental studies on the formation of citric acid by strains of *A. niger* and thereby established the basis for one of the most important of all industrial mold fermentations Subsequent to this investigators in the United States and abroad have made many additional and important contributions While no attempt will be made to cite all of these the works of Bernhauer Wehmer Doelger and Prescott and the U S Department of Agriculture group including Wells May Moyer Herrick and Ward are considered to be outstanding A selected list of references to the citric acid fermentation is presented on pages 290-293

Fumaric Acid Certain strains of the *A. niger* group produce appreciable amounts of fumaric acid and it was to one of these forms that Wehmer in 1918 applied the name *A. fumaricus* At the present time however fumaric acid is produced in industry by fermentation with species of *Rhizopus* rather than strains of the black aspergilli (see p 293)

Gluconic Acid Selected strains of *A. niger* are used industrially for the production of gluconic acid. This process, like the citric fermentation, has been investigated by many workers. Outstanding contributions have been made by Molhard Bernhauer, and the U. S. Department of Agriculture group, including Herrick May, Wells, Moyer, Gastrock, Porges, and others (see pp 295-297).

Oxalic Acid Under certain conditions some strains of *A. niger* produce appreciable quantities of oxalic acid. While it is usually avoided rather than encouraged, this fermentation has been investigated by Wehmer (1891 and 1892) Raistrick and Clark (1919) Jacquot (1938) and others (see pp 298-299).

The production of these various acids in quantities sufficient to have economic importance represents to an appreciable degree specific strain characteristics and the greatest possible care must be exercised in maintaining these strains in a state of high productivity. It has been found however, that in certain cases the same strains can be made to produce substantial yields of two or more of these acids by varying the composition of the nutrient solution and certain environmental factors. The reader is referred to the extensive literature on this subject.

Enzymes While they are not commonly cultivated for the production of enzymes as such as are members of the *A. flavus-oryzae* group, the black aspergilli produce a number of enzymes in appreciable quantities. Beginning with Fernbach in Germany (1890) and Bourquolet (1893) in France, various authors have devoted considerable study to their formation. A number of papers relative to enzyme production by members of the group are cited in the Topical Bibliography under the subtitle 'Enzymes of *Aspergillus niger*' (pp 294-295).

Fat Production The mycelium of *A. niger* contains appreciable fatty materials (Pontillon 1932, Bernhauer and Patzelt 1935 Schmidt 1935 and others). The waste mycelium from the citric acid fermentation is reported to provide a satisfactory source of sterols for irradiation in the production of Vitamin D.

Soil Testing *A. niger* has been successfully employed as an assay organism for determining mineral deficiency of soils particularly deficiencies in phosphorus and potassium. Papers on the so-called *A. niger* method of soil testing were first published by Huesling and Schmidt and by Schlots Smith and Brown in the same year (1932) to be followed by more elaborate studies by Stock (1933) Niklas and associates (1933) and others. Additional references to this method are presented in the Topical Bibliography (pp 313-314). The strain employed by Niklas is maintained in the culture collection of the Northern Regional Research Laboratory as No. 323.

Mildew Strains of *A. niger* represent a common cause of mildew on exposed wood surfaces and cotton fabrics. Partansky and McPherson (1940) used a strain of this species successfully for testing the mold resistant properties of oil paints. *Aspergillus niger* is commonly included in the mixtures of miscellaneous molds used for testing the effectiveness of mildew and rot proofing agents when impregnated in textiles and fabrics. Where pure cultures are employed species of *Chaetomium* and *Metarrhizium* are generally used.

Mold Physiology

Members of the *Aspergillus niger* group have been used extensively in investigations on mold physiology, probably more than any other form. As early as 1909 Latham studied nitrogen assimilation by *A. niger* (*S. nigra*) to be followed in 1911 by Dox studying phosphorus assimilation by the same species. Beginning in 1918 and continuing up to the present time, Steinberg has published a succession of papers on the physiology of *A. niger* with special reference to the rôle of heavy metals in its nutrition. A single strain of *A. niger* which is carried in the NRRL collection as No. 334 (Thom No. 4247) has been used throughout these investigations. Studies of a somewhat similar character have been conducted by Bortels (1927), Levy (1932), Gollmick (1936) and others. Citation of these papers together with many additional references are presented in the Topical Bibliography under the subtitle *Physiology*. An attempt has been made to present sufficient references to serve as a point of entrance to the literature of the field.

Pathogenesis

Members of the *Aspergillus niger* group are commonly isolated from the external ear of man, this being the source of the classic *Sterigmatocystis antracustica* of Cramer. Other species reported to have been isolated from cases of otomycosis include *A. niger* van Tieghem, *A. phoenicis* (Cda) Thom and Church (with long primary sterigmata), *A. giganteus* (Mattliet) Dodge and *A. Macfiei* Dodge. *A. Macfiei* showed no signs of pathogenicity when tested on experimental animals. There is no morphology to distinguish either of the latter two forms from the ubiquitous saprophytic types and it appears probable that many strains can become established in the auditory canal under certain favorable and probably temporary conditions.

Once entrenched in the flesh about the auditory canal the mycelium has been found to be very persistent. One case is known in which occasional abscesses have occurred over a period of 25 years during which desultory treatment has quieted the inflammation but has not destroyed the parasite.

In the same way, extensive development of these forms as points of infection in the lungs may give rise to conditions diagnosed as pseudo-tuberculosis which may persist for long periods without resulting either in death or complete recovery of the patient. Spores of *A. niger* are air borne and hence are commonly drawn into the respiratory tract. They are occasionally reported as causative agents in allergic reactions.

Single Strain Cultures

The great importance of some of the black aspergilli as fermentative agents makes punctilious preservation of the actual strain employed the only means either of insuring a process against breakdown or of introducing it in a new locality. Even when such a culture is maintained with the utmost care natural variation sometimes occurs. Induced variation, such as that described by Steinberg and Thom in a series of papers (1939-40) presents a further hazard in processes where cultures are grown at extreme H ion concentrations, or in the presence of chemical or other substances which may actually be toxic at the concentrations employed. In a process in one laboratory a strain appeared that presented a colony aspect in which only a few typical black heads developed in a background of dwarfed and fractional conidial fruiting structures. In another case a mutant appeared which differed so radically from *A. niger* in its secondary sterigmata and spore chains that the Yuills described it as a new genus and species *Cladosarum olivaceum* (Trans Brit Myc Soc 22 194-200, Pls 11-13 1938).

The user of one of these Aspergilli then, needs to know his organism in cultural aspect, in microscopic details and in essential reactions in standardized and reproducible substrata. Furthermore, he must be able to maintain it free from contamination with other species, and likewise free from such variation, either natural or induced, as will interfere with consistent and controlled results.

CHAPTER XVIII THE ASPERGILLUS WENTII GROUP

Outstanding Characters

Conidial heads large typically globose often splitting irregularly in age—varying in color from dull yellowish to ecru-olive and from light to dark brown depending upon the species and strain
Conidiophores smooth walled or nearly so but often appearing finely roughened when examined in dry mounts
Vesicles globose fertile over the entire surface Sterigmata in two series
Conidia commonly elliptical smooth or somewhat roughened depending upon the species
Sclerotia present or lacking dark brown to black characteristically white tipped when young

The *Aspergillus wentii* group as presented here is recognized as somewhat artificial in comparison with such strictly natural groupings as the *A. glaucus*, *A. nidulans* and *A. clavatus* groups. The degree of relationship between the species included is open to question. Yet all of the forms possess certain characteristics in common: (1) conidiophores are smooth walled or nearly so; (2) conidial heads are large and strictly globose at least when young; and (3) all appear to occupy taxonomic positions somewhat intermediate between the *Aspergillus niger* group on the one hand and the *Aspergillus flavus* or *Aspergillus ochraceus* groups on the other.

Group Key

I Conidia smooth walled
A Sclerotia lacking vegetative mycelium and young conidiophores reddish in color
1 Conidial heads light brown near wood brown (Ridgway Pl. XL)

B Sclerotia present dark brown to black vegetative mycelium colorless and young conidiophores colorless or pale yellow
1 Conidial heads dull yellow to ochraceous sclerotia globose or nearly so

2 Conidial heads in yellow green shades near ecru-olive (Ridgway Pl. LXX)
A *alliiaceus* Thom and Church
A *arenaceus* G. Saut.

II Conidia more or less echinulate
A Sclerotia present or lacking depending upon the strain and the substratum conidial areas in orange brown to brown colors
1 Conidiophores colorless colonies often conspicuously floccose
2 Conidiophores brown (See *A. niger*)
A *wentii* Wehmer
A *hennebergi* Blochwitz

Aspergillus panamensis Raper and Thom in *Mycologia* 36 568-572
fig 5 1944

Colonies on Czapek's solution agar at room temperature very thin consisting of a sparse and transparent growth of vegetative hyphae almost wholly submerged bearing widely scattered erect conidial structures with radiate heads light brown in color Colonies upon malt extract agar at room temperature growing well and fruiting luxuriantly reddish brown in color, consisting of a dense basal mycelium, predominantly red, from which develop massed conidial structures in broken or continuous concentric zones (fig 65 A) many conidiophores abortive and sterile, fertile conidiophores bearing globose to radiate heads light brown in color, near wood brown (Ridgway, Pl XL), these, together with red-colored sterile structures and aerial hyphae give the colony its characteristic appearance and color, in age, colonies tending to develop a loose floccose overgrowth, more or less obscuring the abundant conidial heads reverse dull brown odor none

Conidial structures arising directly from the substratum scattered or abundant, depending upon the culture medium employed (fig 65 B) Heads typically globose in age characterized by loosely radiating chains of conidia, less commonly by few to several roughly columnar masses variable in size, commonly ranging from 250 to 450 μ in diameter occasionally up to 500 μ varying in color from avellaneous to wood brown (Ridgway, Pl XL) to Saccardo's umber (Ridgway, Pl XXIV) Conidiophores straight mostly 600 to 900 μ in length by 9 to 12 μ in diameter occasionally larger, with walls smooth, comparatively heavy, ranging from 3 to 3.5 μ thick in the basal area to 1.5 to 2 μ in the terminal area approximately uniform in diameter throughout except for a limited reduction immediately beneath the vesicle Vesicle colorless comparatively thin walled globose or slightly elongate mostly 25 to 30 μ in diameter fertile over the entire area (fig 65 C) Sterigmata in two series closely packed primaries 5.5 to 6.5 μ by 2.4 to 2.8 μ secondaries 5 to 6 μ by 1.5 to 2 μ Conidia light yellowish brown in mass globose to subglobose smooth walled mostly 2.2 to 2.6 μ in diameter occasionally 2.8 μ

Type culture NRRL No 1785 was isolated in January 1942 from Panama soil collected by Mr John T Bonner A second culture NRRL No 1786 differs from the above strain in minor details but clearly belongs with it This was isolated from a second sample of Panama soil collected by Bonner

The species is considered to represent a form somewhat intermediate between the *Aspergillus niger* group and *A. wentii* Superficially at least, there is evidence of relationship with *Aspergillus niger* mut. *cinnamomeus* (syn *Aspergillus cinnamomeus* Schiemann) and *Aspergillus niger* mut. *Schiemannii* (syn *A. fuscus* Schiemann) It bears a certain

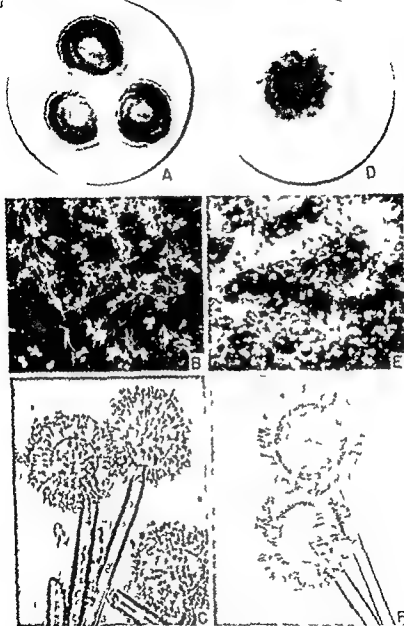


FIG. 85. A-C *Aspergillus panamensis* NRRL No. 178. A Colonies upon malt extract agar 10 days. B Conidial heads showing tendency to split into loose divergent columns $\times 500$. C Conidial heads showing globose vesicles and sterigmata in two series $\times 500$. D-F *Aspergillus niger* NRRL No. 17. D Single colony on Czapek's solution agar showing numerous large sclerotia. E Portion of above colony showing greater detail of heads and sclerotia $\times 500$. F Conidial heads showing large globose vesicles $\times 160$.

resemblance to these forms in the comparatively light color of its conidial heads and in the smallness and general character of its conidia, but differs from these forms in three very striking particulars (1) It characteristically develops an extensive red colored aerial mycelium upon media such as malt-extract agar where it attains its maximum growth, (2) it grows very sparsely upon Czapek's solution agar upon which the above noted forms grow luxuriantly, and (3) it possesses very small primary sterigmata, measuring 5.5 to 6.5 μ by 4 to 2.8 μ in contrast to 13 to 15 μ by 3 to 5 μ for mut *cinnamomeus* and 15 to 40 μ by 4.6 μ for mut *Schicmanni*. Whether or not the species actually represents a naturally occurring mutation from *A. niger* can only be guessed. The smallness of its conidia and primary sterigmata would hardly support this hypothesis. In cases where mutations have been obtained from known cultures the dimension of specific structures in such mutations generally agree very closely with those of the same structures in the parent strain, and black *Aspergilli* with the dimensions of *A. panamensis* are rarely, if ever, encountered in nature. The possibility of this representing a mutation is not excluded, but until additional evidence supporting such origin is forthcoming the writers feel warranted in maintaining as a distinct species this unique form which obviously is able to maintain itself in the soils of Panama.

The correct taxonomic position of this species remains in doubt. It is included with *Aspergillus uentii* although we realize that this placement is not entirely satisfactory. As continued isolations are made from tropical soils and other sources it is our hope that additional forms may be found, which will furnish evidence of a more exact relationship.

The very sparse development of *A. panamensis* upon Czapek's solution agar containing sucrose results from an invertase deficiency, when dextrose is substituted as a carbon source the fungus grows luxuriantly and fruits abundantly.

Aspergillus alliaceus Thom and Church, in *The Aspergilli* p 163 1926

Discussed without name as Thom No. 4660 by Walker and Lindgren, in *Jour Agr Res* 29 507-514 1921, and by Walker, Lindgren, and Bachmann, in *Jour Agr Res* 30 175-187 1925

Colonies on Czapek's solution agar rapidly and broadly spreading with loosely floccose aerial sterile mycelium bearing scattered ochraceous heads among abundant dark to almost black sclerotia (Pl. VI D and fig. 66 A) in some strains, predominantly floccose with limited conidial heads and few sclerotia in others (fig. 66 B) reverse uncolored. Conidial heads dull yellow to ochraceous strictly globose when young and remaining radiate or splitting irregularly in age (fig. 66 D) up to 300 μ in diameter often more abundant in cultures after many transfers. Conidiophores up to 150 μ

long by 15μ with walls up to 1.5μ in thickness appearing smooth in liquid mounts but showing rudimentary markings or pits when examined dry. Vesicles globose to subglobose up to 40 to 50μ in diameter, with walls about 2μ in thickness and showing pores where sterigmata are attached. Sterigmata in two series, primary 7 to 9μ or even 12μ by 3 to 4μ secondary

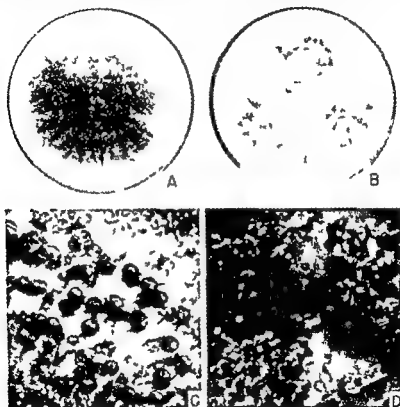


FIG. 66 *Aspergillus alliaceus*. A Heavy sclerotium producing strain NRRL No 315, on Czapek's solution agar 10 days. B Light sclerotium producing strain NRRL No 318 growing under similar condition. C Portion of A showing greater detail of sclerotia and conical heads $\times 9$. D Coridial heads from old culture (strain NRRL No 318) showing tendency to split into divergent columns $\times 18$.

7 to 8μ by 2μ . Conidia elliptical to globose, yellowish 2.5 or 3μ in diameter. Perithecia not found. Sclerotia often very abundant (fig. 66 C) at first white but quickly becoming black or nearly so in many strains dominating the character of the colony.

Strains compared include NRRL No 315 (Thom No 4656) from a dead

blister beetle (*Macrobasis albida*), NRRL No 316 (Thom No 4660) isolated from onion bulbs and others with characteristic heads and sclerotia have been obtained from garlic bulbs from cactus plants and from soils particularly from the general region of Texas, Arizona and Mexico. Strains have also been isolated from soils collected near Calcutta.

The large globose, pale yellow to ochraceous heads of the species strongly suggest relationship to the 4 *ochraceus* group. The smooth walled conidiophores and black sclerotia however, more closely ally it with *Aspergillus wentii* and the other species grouped with it. It may however represent a form somewhat transitional between the great groups represented by *A. niger* on the one hand and *A. ochraceus* on the other.

Aspergillus alienaceus Geo Smith in Brit Mycol Soc Trans 25 24-27, Pl 1, figs 1-3 1943

Colonies on Czapek's solution agar (with sucrose) at room temperature spreading rapidly, more or less conspicuously zonate (fig 65 D), slightly floccose white at first then dull yellow to ecru-olive (Ridgway, Pl XXX) as shown in Pl VI E, with, at times, a greenish tinge without becoming truly green, reverse pale dirty pink. Conidial heads large globose 400 to 600 μ in diameter, or up to 1000 μ , splitting into columnar masses of conidial chains. Conidiophores up to 5 mm long 18 to 30 μ in diameter with walls 2.5 to 4 μ thick, smooth in fluid mounts, but appearing finely roughened when examined dry. Vesicles globose or slightly flattened thick walled up to 185 μ in long axis sterigmata in two series primary 22 to 50 μ by 6 μ secondary 11 to 13 μ by 4 μ (fig 65 F). Conidia ellipsoid smooth 4 to 6 or 6.5 μ by 3.2 to 4 μ . Sclerotia dark grayish brown to black (fig 65 E) elongate irregularly flask shaped sometimes with the neck forked, apical portion white to gray during development 2 to 3 mm in long axis scattered in concentric zones after 7 to 10 days.

On Czapek agar with glucose, sclerotia are more abundant and larger. On wort or potato agar, conidial heads are abundantly produced but sclerotia are delayed for several weeks and are few in number.

Species characterization adapted from George Smith's description.

This very distinctive species (NRRL 517 Thom 572c) is represented by a single isolation from seed peas made in 1938 by Dr G. E. Turfitt of the London School of Hygiene and Tropical Medicine University of London.

Aspergillus wentii Wehmer in Centralbl f Bakt etc 2 Abt III p 150 1896. See also The Aspergilli Thom and Church p 183 1926.

Colonies on Czapek's solution agar rapidly growing and broadly spreading floccose with white or yellowish aerial hyphae which in some strains pile up in the plate (Pl VI F and fig 67 A) or fill the test tubes for several

centimeters (fig 67 C) but remain inconspicuous in other strains (fig 67 B), with developing heads at first white through yellow shades to olive brown medial bronze or snuff brown (Ridgway Pls IV XVI, XX column 19, and XXII column 15 h) or according to Wehmer, coffee brown to chocolate brown reverse becoming reddish brown in old cultures. Conidial heads large globose generally remaining radiate in age (fig 67 D) ranging up to 500μ in diameter, changing from yellow shades to brown. Conidiophores up to several millimeters in height by 10 to 25μ in diameter with walls colorless up to 4μ in thickness studded with droplets in growing colonies and often appearing slightly roughened when examined dry, but uniformly smooth in fluid mounts. Vesicles globose or nearly so (fig 67 E) varying up to 80μ in diameter fertile over the entire surface. Sterigmata usually in two series primaries 10 to 20μ by 3 to 5μ occasionally much larger secondaries 6 to 8μ by 3μ . Conidia borne in long chains more or less elliptical ranging from 3μ to 6μ in long axis but mostly 4 to 5μ double wall clearly evident ranging from almost smooth to marked by ridges sometimes suggestive of *A. niger* again more closely resembling the *A. flavus* series. No perithecia reported. Sclerotia often encountered (fig 67 F) dark brown to black ovate with long axis vertical.

Culture description based upon strain NRRL No 375 (Thom No 116) obtained in 1909 from the Centraalbureau as Wehmer's original organism as well as numerous isolations from soils and other materials collected by the authors in the United States and other strains contributed by investigators from all over the world.

Numerous strains with the general aspect of Wehmer's species have been seen from Java China South America Japan the Straits Settlements British Guiana and Brazil. In our experience it has been isolated from cottonseed cake from olives from soil and from numerous other sources. It is to be regarded as very widely distributed and to be common on many types of decaying vegetable products.

The variations in colony aspect in different strains run from an extreme of mycelial growth filling the test tube that is characteristic of cultures such as the Wehmer organism to colonies forming a crowded surface growth of conidiophores only and distinguishable from *A. tamarii* only by a lack of greenish color in the early fruiting period and in the characteristic smooth conidiophores and finely roughened conidia.

Aspergillus archaeoflavus Blochwitz (Ann Mycol 31(1/2) 73, 1933) represents a non floccose form which is hardly separable from *A. wentii*. In our examination of the type strain (NRRL No 382 Thom No 5346) received in 1933 from Baarn measurements were somewhat less than those cited by the author. The absence of conidial markings to which Blochwitz called attention would not bar it from *A. wentii* since in some strains conidia are almost entirely smooth in others finely roughened while in still others they are conspicuously echinulate.

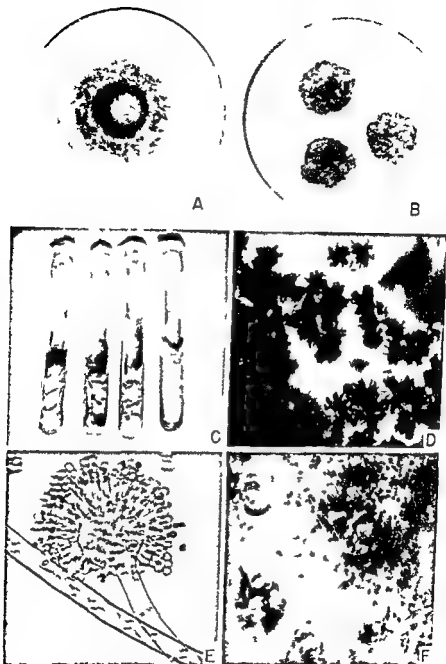


FIG. 67. *Aspergillus wentii*. A and B colonies of strains NRRL No. 375 and No. 385 respectively upon Czapek's solution agar 10 days. C Tube cultures of four representative strains. D Conidial heads from old culture $\times 18$. E Single head showing smooth conidiophore, globose vesicle and sterigmata in two series $\times 500$. F Detail of colony margin in strain NRRL No. 379 showing sclerotia and abundant conidial heads $\times 9$.

- Aspergillus wentii* var *minimus* Nakazawa Takeda Okada and Sino (Jour Agr Chem Soc Japan 10(3) 148-177 1934) shows measurements varying somewhat from those of the species and from those given by Blochwitz but it is not sufficiently marked to warrant separation.
- Sterigmatocystis orrea* Baier (Bull Soc Bot France 27 28 1881) may have been a member of this series but was not sufficiently described to permit positive identification.
- Aspergillus kennedyeri* Blochwitz in Ann Mycol 33 235-239 1935 The species is described as having the aspect and colors of a non floccose *A. wentii* or an *A. lamarii* with red sclerotia but with conidiophores browned as in the partially browned conidiophores of the *A. niger* group.
- An albino variant of *A. wentii* was isolated by Moneray (Ann Soc Sci Brux 84 181 189 1934) from a normal culture of this species and was found to retain its distinctive characters through repeated transfers in laboratory culture. No name was given to this mutant.

Occurrence and Economic Importance

Aspergillus wentii is a cosmopolitan species that is fairly common in soils upon moist grains and other vegetable matter undergoing slow decomposition and may be isolated less frequently from a wide variety of other materials collected from nature. It is apparently world wide in distribution. In the Orient it is often included with *Aspergillus tamarii*, *A. flavus* and *A. oryzae* all under the latter name as a rule in the 'Koji' preparations used in the manufacture of various soy products. Lakenice it has been investigated with these same species in connection with the production of various mold enzymes. At the same time it has been included with the black *Aspergilli* in studies on the production of organic acids by molds. Recently Larson (1942) has reported one strain of this species to give substantial yields of citric acid in submerged culture. Yabuta (1912) reports Koji acid production by *A. wentii*. On the whole neither the black *Aspergilli* or members of the *A. flavus-oryzae* group. It is nevertheless a vigorously growing species with definite biochemical possibilities and should not be overlooked in any program relating to mold fermentation.

Aspergillus alliacerus appears periodically upon alliaceous bulbs and occasionally upon cacti as at least a secondary parasite. It is not infrequently isolated from soils and appears to be fairly common in the southwestern states of Texas and appears to be fairly common in the southern states of other areas including Southern Mexico and India. Nothing is known regarding its biochemical possibilities. Only the type strains of *A. aculeatus* and *A. panamensis* are known and neither has been shown to have any economic importance.

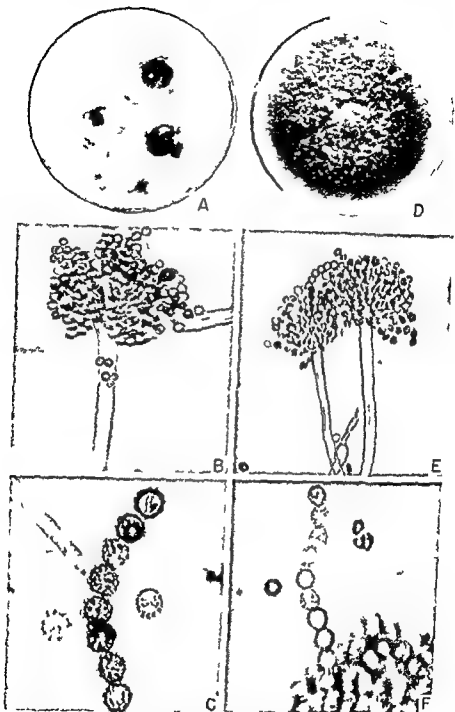


FIG 85 A-C *Aspergillus luteascens* NRRL No. 426 A Colonies on Crapek's solution agar showing extreme floccose habit and light sporulation 10 days B Single conidial head $\times 500$ C Conidia $\times 1200$ D-F *Aspergillus terricola* NRRL 424 D Single colony on Crapek's solution agar showing somewhat floccose but heavily sporing colony 10 days E Conidial heads showing sterigmata in a single series $\times 500$ F Conidia $\times 1200$

in one series in smaller and crowded heads up to 15 to 20μ by 4 to 5μ sterigmata often in two series in larger heads with primaries about 15 to 18μ by 4 to 5μ secondaries 12 to 14μ by 4 to 5μ Conidia subglobose varying from 5 by 7μ to 8 by 9μ conspicuously roughened with prominent tubercles of color (fig 68 C)

The species is known only in the type culture from the Bamier collection NRRL No 425 (Thom No 4640-478) and as a second strain NRRL No 426 isolated in the Soil Microbiology Laboratory Bureau of Plant Industry Washington D C about 1939

Aspergillus terricola Marchal in Rev Mycologique 15 No 59 101-103 1893

Colonies umbrinus mycelial hyphae 3 to 5μ in diameter without anastomoses conidiophores hyaline continuous or septate in age 600 to 1000 μ by 7 to 10μ (whole depth of colony growth) vesicles subglobose hyaline 39 to 50μ radiately covered with sterigmata sterigmata in one series 12 to 18μ by 4 to 7μ conidia umber (Sacc) ovate or elliptical then globose rough with colorless connectives

Description from Marchal of a culture isolated from soil in Belgium not reported elsewhere see variety below

Aspergillus terricola var *americana* Marchal cultural description by Thom and Church in Am Jour Bot 8 125 1921

Colonies on Czapek's solution agar growing rapidly at room temperature often somewhat floccose in central colony areas (fig 68 D) ranging from shades near yellow ochre (Ridgway Pl VI) when young to Dresden brown or mummy brown in age near Saccardo's umbrinus (Pl VII A) aerial growth largely consisting of crowded conidiophores reverse uncolored Conidial heads radiate hemispherical to subglobose loose in texture consisting of comparatively few divergent chains of conidia up to 200μ in diameter Conidiophores 300 to 600μ in length by 6 to 8μ in diameter with walls pitted Vesicles globose to subglobose (fig 68 E) up to 20μ in diameter fertile over the upper two thirds or three fourths Sterigmata in one series 7 to 10μ by 2 to 4μ Conidia tuberculate (fig 68 F) from the presence of color bars variously distributed between the outer and inner wall ovate to nearly globose from 3 by 5μ up to 5 by 7μ usually about 5.5μ occasionally 5 to 8μ in diameter

Type culture NRRL No 424 (Thom No 4838) isolated by F M Scales from redland soil in Georgia and discussed by Scales in Jour Biol Chem 19 459-472 1914 under the name *A. terricola* Marchal Scales culture was submitted to Marchal who designated the form as *A. terricola* var *americana* Marchal distinguished as follows The dimensions of the

vesicles 14 to 20 μ instead of 30 to 50 μ of the sterigmata 5.6 to 10.5 μ by 2.2 μ instead of 12 to 15 μ by 4 to 7 μ the spores only very delicately verrucose, separate your fungus from *A. terricola*!

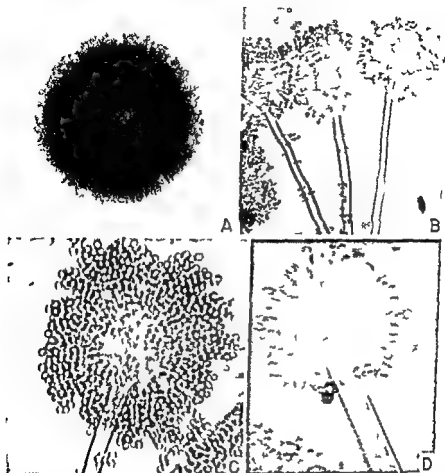


FIG 69 *Aspergillus tamaris*. A Colony growing on Czapek's solution agar characterized by heavy conidial production strain NRRI No 427 10 days B, Conidial heads $\times 160$ C Single head $\times 300$ D Single head further enlarged showing rough walls of conidiophore and sterigmata in two series $\times 500$

Aspergillus tamaris Kita in Centralb f Bakt etc 2 Abt 37, No 17/21, pp 433-452 1913 Characterization of *A. tamaris* Kita given by Thom and Church in Am Jour Bot 8 118 1921 See also Thom and Church The Aspergilli p 191 1926

Colonies on Czapek's solution agar spreading broadly at room temperature (fig 69 A) with vegetative hyphae mostly submerged fruiting areas

at first colorless then passing through orange yellow shades to brown in old colonies variously Isabella color light brownish-olive buffy-citrine medial bronze or mahogany (Pl. VII B) (Ridgway Pls. VII, XII and XIII) presenting a suggestion of green that is transient and limited to areas of young heads reverse uncolored or occasionally pinkish. Conidial heads varying greatly in size in the same fruiting area from more or less columnar to nearly but not completely globose and up to 300 μ in diameter with radiating chains and columns of conidia. Conidiophores arising from submerged hyphae up to 1 to 2 mm in length colorless with walls becoming abruptly thinner at the base of the vesicle frequently showing irregular thickenings within as a rule markedly rough or pitted throughout part or all of their length (fig. 69 D) sometimes appearing smooth or nearly so when examined in liquid mounts but consistently rough or pitted when examined dry. Vesicles globose to subglobose 2 μ to 50 μ in diameter (fig. 69 C) with fairly thin walls which frequently crush in mounts fertile over almost the entire surface. Sterigmata in one series in small heads in two series in large heads primary sterigmata commonly 7 to 10 μ by 3 to 10 μ becoming 20 to 35 μ long in gigantic heads secondary sterigmata 7 to 10 μ by 3 μ . Conidia ranging from more or less pyriform through subglobose to globose conspicuously roughened from prominent tubercles and bars of orange yellow coloring matter deposited between the loose outer wall and the firm inner wall commonly ranging from 5.0 to 6.5 μ in diameter or reddish purple globose to pyriform produced by many strains usually purplish.

Species characterization is based upon Thom and Church's culture No. 4235 12 (ARRL No. 429) which was submitted to and identified by Kita as *A. tamarii* (see Thom and Church *Am. Jour. Bot.* 8: 118, 1921).

The organism described by Kita proved to be one of a great series represented in our collection. It is common among cultures examined from North and South America from Japan, China, India and from Europe. The species has been found to be quite common in soil collected from many areas in the United States.

The outstanding characters are orange yellow to brown colonies coarse colorless conidiophores usually roughened but with these characters sometimes obscure large radiate loose textured conidial heads sterigmata in one or two series commonly with single and double sterigmata in the same head conidia more or less pyriform 5 to 8 μ in long axis with tubercles or bars of orange yellow coloring matter between the inner and the outer cell walls. In preparing the manuscript for *The Aspergilli* (1926) Thom and Church introduced into their general key without name on page 248 under No. 279 a series of forms with morphology and general appearance

bridging the gap between *A. tamaris* Kita and the *A. flavus* group. Without publication they referred to these as 'The Bronze Series'. These strains have the yellow green color of *A. flavus* during the early stages of their development, but subsequently develop the yellow to brown colors of *A. tamaris*. Recognition of this border group is necessary since some strains of *A. tamaris* do not assume a definitely green color at any state in their development, while others show green as a transient character. Strains of *A. flavus*, on the other hand, are typically characterized by the green to yellow green colors. Furthermore, the conidia of *A. tamaris* are typically quite roughened, showing prominent tubercles or bars of coloring matter deposited between the outer and inner walls. In contrast, the conidia of *A. flavus* are less coarsely roughened and show more numerous and smaller tubercles or echinulations as well as a greater tendency for the coloring substance to be generally diffused throughout the spore envelope. The forms under consideration show in some degree the coloration and spore characters of both groups and are believed to be truly intermediate between *A. tamaris* and *A. flavus*.

The fact that we received from Baarn in 1933 as Blochwitz's *A. luteo-virescens* Bloch (Ann Mycol 31 73-83 1933) a culture (Thom No 5345) which represented satisfactorily this intermediate series is not accepted as justifying the assignment of this name to the series since the morphological characters displayed by the strain were so completely at variance with the original description, and since Blochwitz considered his species to be close to *A. ustus*. We question whether any sharp line of separation can be drawn between the two series because of the repeated appearance of intermediate forms. While we do not feel justified in assigning to these forms any specific designation we do feel obligated to continue to call attention to their existence. We have at times considered the desirability of moving the whole *A. tamaris* complex over into the *A. flavus-oryzae* group, but this course has been abandoned since it was felt that to do so would introduce into an otherwise perfectly integrated group a series of organisms whose relationship to them while strongly suggested is not proved and which in its typical form would introduce discordant features.

The species listed below are believed to represent probable synonyms.

Bourge attached the manuscript name *A. vulpinus* to a member of the *A. tamaris* series (Thom No 4733 146) and contributed it to our collection but it does not seem sufficiently different from the species to warrant separation.

One strain of *A. tamaris* was found in the Baunier collection (Thom No 4640 397) as *A. cacao* nomen nudum another under the same name came from Pribram.

A. gigas Spegazzini Myc Argent V in An Mus Sac Buenos Aires Ser B Tome 13 424 1911 was described from decaying coffee leaves in terms that suggest its relationship to *A. tamaris*.

A. spadix Amons in Archief voor de Suikerindustrie in Nederlandsch Indie Jaarg 29 Deel 1 pp 12 14 Jan-June 1971 From the description this is a synonym of *A. tamaris* Kita Colonies described as yellow brown to deep brown growing well on common laboratory media without aerial mycelium in reverse colorless rice colored to light violet at first then light fuscous brown conidiophores up to 2 to 3 mm by 8 to 9 μ with walls about 0.9 μ thick and pitted or rough vesicles globose up to 50 μ in diameter or almost clavate in small heads conidia 5.5 to 7.2 μ rough Culture Amons Not studied by us

A. erythrocephalus B and C in Jour Linn Soc (London) Bot 10 382 1869 (See Fungi Cubensis Wrightiana 1868 Type No 647 in Curtis Herbarium deposited in the Cryptogamic Herbarium of Harvard University bears Wright's No 784 Part of the original material was removed by Dr Farlow and given to Thomas for study)

Microscopic examination of this type specimen gives measurements as follows conidiophores 45 to 70 μ in diameter up to 2 mm in length with walls very heavy 8 to 12 μ thick varying from 5 to 6 μ in the broader part to 10 to 12 μ at the narrower base pitted or roughened vesicles up to 100 μ in diameter nearly globose fertile all over head washed free from spores about 150 μ in diameter sterigmata in two series primary 8 to 10 μ in length secondary 8 to 9 μ in length conidia commonly 8 by 6 μ ranging up to 8 to 12 μ by 5 to 9 μ finely pitted or roughened with rather thin walls Colors in the material are questionable on account of the age of the collection

Cultures None Type material only known Placed between the *A. tamaris* and *A. flauus* groups The amended description is offered due to the existence of a type specimen with very conspicuous characters under a name only very briefly described in 1869 When grown upon natural substrata such as grains et cetera conidiophores and heads of *A. tamaris* become very much larger than those ordinarily produced in culture media This might account for this specimen which bears the name *A. erythrocephalus* B and C

Occurrence and Economic Importance

Of the species included in this group of brown spored *Aspergilli* only *A. tamaris* is in any sense widely distributed or common in nature *Aspergillus lutescens* is known only as the type culture and as a second isolation made in Washington D C many years later *Aspergillus terricola* has not been positively identified since its description although the form with smaller heads and less coarsely roughened spores designated *A. terricola* var *americana* by Marchal is occasionally encountered *Aspergillus tamaris* is however a cosmopolitan mold upon vegetable material undergoing slow decomposition and can be isolated from almost all soils examined like *A. niger* and *A. flauus* it is more frequently recovered from warm and semi tropical soils than from cool temperate soils although it occurs in the latter The species commonly appears with *A. flauus* and *A. oryzae* as a constituent part of the koji used in the fermentation industries of the Orient Certain strains apparently produce appreciable amounts of diastatic and proteolytic enzyme while other strains are known to produce

kojic acid Gould reported this in 1938 and it was subsequently confirmed by A. J. Moyer (unpublished notes) for a strain isolated from Panama soil at the Northern Regional Research Laboratory in 1941.

Kita's culture was isolated from a soybean sauce termed "Tamar", hence the species name. Tamar is made by a shorter fermentation process than soy sauce or shoyu, and differs from it in flavor. Kita believed that where it was made empirically it owed its individuality to the particular aspergillus which he isolated and described.

CHAPTER XX THE ASPERGILLUS FLAVUS ORYZAE GROUP

Outstanding Characters

- Colonies varying from very light greenish yellow to deep yellow green (Ivy Green)
- Conidiophores rough or pitted colorless
- Heads hemispherical to columnar often varying in the same head
- Sterigmata in one or two series from hemispherical to dome shaped in small heads to globose in large heads
- Vesicles variable in form varying in color as the colony
- Conidia more or less roughened generally grayish brown to black entirely lacking in others

Two species names are widely used for members of this cosmopolitan group *Aspergillus oryzae* is applied quite generally without regard to morphology to the strains used by the Japanese and Chinese in the fermentation of rice and soy products. Although purified cultures are used in many places the nomenclature is based more upon utilization than upon morphology. There appears however in these industries a series of strains with long conidiophores radiate heads mostly greenish yellow with the green often fading completely in old cultures. These strains appear to be most commonly used in the production of the diastatic type of ferments and to be distributed in the great culture collections as *Aspergillus oryzae* (Ahlb.) Cohn. Such strains seem to be mostly oriental or tropical in origin. Strains with shorter conidiophores and yellowish green heads on the other hand appear wherever fermenting or decaying materials are examined microscopically or by culture. *Aspergillus flavus* Link has been accepted as a species aggregate for this second array of forms from which the segregation of sections for description as separate species has been found difficult if not almost impossible. If one wishes to perpetuate species names as roughly covering aggregates of closely related but varying strains bearing always in mind that no sharp lines of differentiation exist certain applications of names may be made arbitrarily about as follows

Group Key

- 1 Sterigmata mostly in one series double sterigmata also present
- A Conidiophores long 1 several mm heads radiate greenish yellow conidia pyriform more or less roughened variable in size up to 6 8 or even 10µ in long axis
- A *oryzae* (Ahlburg) Cohn

- II Conidiophores 600 to 1700 μ heads radiate hemispherical pale greenish yellow conidia smooth globose 3.0 to 4.6 μ *A. micro virido-citrinus* Costantin and Lucet
- C Conidiophores mostly less than 500 μ heads deep yellowish green (Ivy Green) described as a parasite of the mealy bug of cane occasionally elsewhere *A. parasiticus* Spegare
- II Sterigmata mostly in two series but single series common and often in same head small heads usually showing single series only
 - A Conidiophores very variable in length mostly 400 to 1000 μ heads in various yellowish green shades *A. flavus* Link
 - II Conidiophores mostly borne as short branches from trailing hyphae forming an uneven cottony mass heads white to yellow with traces of green only *A. effusus* Tiraboschi

Literally hundreds of strains of this group have been collected and compared. Many of them have been isolated from fermentation investigations in the laboratory and from industrial processes. No correlation of colony appearance, conidiophore or head morphology, color or microscopic detail, with actual utilization has been proved. A culture labeled 4 *oryzae* (Ahlburg) Cohn, NRRL No. 447 (Thom No. 113) has been preserved for over 30 years without apparent change in morphology. It is probably derived from Cohn's organism. When however we scrutinize the *Aspergilli* obtained from the rice or soy fermentations of the Orient cultures of the type represented by this strain are not the most common. The pre-eminently useful strains usually have the aspect of forms intermediate between *A. flavus* and *A. oryzae*. The dwarf green *A. parasiticus* of Spegare isolated from dead mealy bugs of sugar cane in Hawaii proved no more parasitic to the same species of insects in the Barbados than other 1 *flavus* strains sent with it. Teizo Takahashi contributed his series of strains under the letters used in his publication (1913). These are discussed at some length in Thom and Church's paper on *A. flavus*, *A. oryzae* and associated species (1921) and also in 'The *Aspergilli*' (1926 p. 202). It is sufficient to say that they vary all the way from almost white with few lightly colored heads to rich yellow green in which heads are very numerous and fairly dark. They vary likewise in the length and diameter of their conidiophores. Characters of color and conidiophore length are not always correlated, although it is generally true that the darker conidial masses are borne upon shorter stalks. The collections contributed by Oshuma Kita Hanzawa and others from Japan as well as those isolated from commercial koji (sold as inoculum for fermentation industries) showed mainly the 1 *flavus* morphology. Strains of this series appear constantly where cultures are made from soil or from decaying vegetation. *A. flavus* and its allies appear in collections from every correspondent who contributes *Aspergilli*. It is debatable whether the worker will be benefited or confused by the introduc-

tion of some of the species names applied to members of the group. It must not be forgotten that any variant from the dwarf and deep green *A. parvulus* to the longest stalked and palest greenish yellow *A. oryzae* may be found if we look for it.

Aspergillus oryzae (Ahlburg) Cohn in Jahresb. Schles. Gesell. Vaterl. Cultur (1183) 61. 226. Breslau 1884.
 The name *E. oryzae* with an incomplete description for the sake of the organism was published by Korschelt in Dingler's Polytechnisches Jour. 230. 330. 1878 as taken from a letter from Herr Ahlburg. See also Thom and Church Amer. Jour. Bot. 8. 106. 1921 and The Aspergillus p. 193. 1926.

Colonies on Czapek's solution agar rapidly spreading with vegetative hyphae mostly submerged and forming a white to gray mycelial layer in the form of a tough felted mass (fig. 70 A) developing pale greenish yellow shades with the production of ripening conidial areas varying from lime green to mignonette green (Ridgway Pl. XXXI column 25) with the green disappearing later and the general color shifting to yellowish brown shades mycelium and agar uncolored. Conidial heads predominantly large abundant globose radiate with chains of conidia separate rather than adhering (fig. 70 D) giving the pale yellow shades of the colonies. Conidiophores 2 to several mm long by up to 20 to 25 μ in diameter with walls rather thin definitely pitted or rough (fig. 70 F) colorless. Vesicles globose to subglobose less often hemispherical up to 50 or even 70 μ with walls 1 to 1.5 μ series with primary sterigmata up to 12 by 5 μ and secondary sterigmata 10 to 12 μ by 3.5 μ (fig. 70 B). Conidia more or less pyriform (fig. 70 F) vary greatly in size in the same culture and in different strains 3 by 4 μ 4 by 5 μ 5 by 6 μ or up to 9 μ or 10 μ in long axis occasionally rather thin walled roughened becoming coarsely and deeply roughened in some strains. Sclerotia dark few and not forming clumps produced sporadically under undefined conditions.

Diagnosis based primarily on culture NRRL No. 447 (Thom No. 113) received from the Centralbureau at Baarn and believed to be derived from Cohn's original strain.

While the above description is believed to conform closely to the original conception of *A. oryzae* strains possessing the essential morphology described but which are heavier sporing and somewhat darker in color are more commonly encountered. Culture NRRL No. 458 obtained from Dr. Oshima as strain AoOld and shown in Pl. VII C and Fig. 70 C-F is representative of these forms.

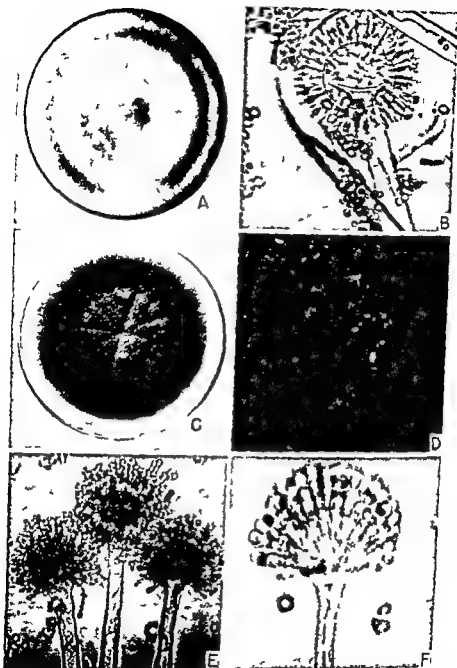


FIG 70 *Aspergillus oryzae* A and B Strain NRRL No 447 (Thom No 113). A Single colony on Czapek's solution agar loose textured conidiophores long and limited in number 10 days B Details of single head vesicle thin walled sterigmata mostly in two series $\times 480$ C-F *Aspergillus oryzae* NRRL No 458 C, single colony on Czapek's solution agar comparatively heavy sporing 10 days D Conidial heads of the same $\times 111$ E Conidial heads further enlarged $\times 270$ F single head showing vesicle sterigmata in a single series and roughened conidiophore $\times 775$

The production of perithecia by members of this series was reported by Bezssonoff (1919) without adequate description and by Zikes (1922) whose culture as received from him belonged in the *A. glaucus* group. No ascosporic form is verifiable for the group thus far.

Aspergillus micro-citrino-citrinus Constantin and Lucet in Ann Sci Nat Bot (IV) 2 158 1905

The appearance of colonies and measurements of conidiophores heads and spores indicate a form intermediate between *A. flavus* and *A. oryzae* except for its small conidia. The description is very nearly satisfied by Takahashi's culture P (NRRL No 480). It was found to grow between 15 and 45 C and to be pathogenic to rabbits. Colonies were greenish yellow to predominantly yellow but contained some definitely green admixture in contrast to *A. oryzae* which often lacks green color entirely. Conidiophores 600 to 1700 μ in length up to 21 μ in diameter near the vesicle uncolored granular (= pitted) above smooth toward the base. Vesicles 24 to 62 μ in diameter. Sterigmata varying in size and arrangement with the size of the heads examined. Conidia globose smooth 3 to 4.6 μ (3.1 μ as a minimum to occasional diameters of 5.5 μ). An occasional culture shows the morphological characters described by Constantin and Lucet. No actual identity has been proved.

Aspergillus flavus Link in Obs p 16 1809 also in Sp Plant 6 66 1824 cited as synonym of *Uromyces flava* Persoon Myc 1 p 30

Synonym *Uromyces Aspergillus flavus* DeBary and Woronin in Beitrage zur Morphologie und Physiologie der Pilze III Reihe p 380 1870. Examined by Brefeld preserved in Rabenhorst Fungi Europaei Edit Nov ser II No 2135 one packet in the collection of the New York Botanical Garden.

Colonies on Czapek's solution agar spreading rapidly with floccosity limited to scanty growth of sterile hyphae in older and dryer areas among crowded conidiophores. conidial areas range in color in various strains from sea foam yellow through chartreuse yellow citron green lime green to Kronberg's green (Pl VII D and fig 72 A) or even to ivy green (See Ridgway Pl XXXI column 25) yellow green colors are either persistent or in old colonies altered by the disappearance of the green factor leaving shades of yellow brown reverse yellowish at first passing over into brown shades in age. Conidial heads vary from small with a few chains of conidia to large radiate (fig 72 E) or columnar masses in the same culture and varying mixtures of different types and sizes of head. Conidiophores mostly arising from submerged hyphae commonly 400 to 1000 μ long by 5 to 15 μ in diam.

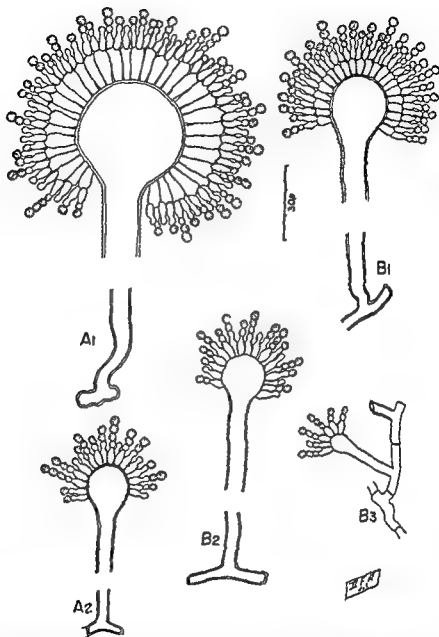


FIG. 71. Conidial structures in the *Aspergillus flavus-oryza* group. *A* *A. flavus* NRRL No. 482. *A*₁, typical large radiate to globose head showing sterigmata in two series. *A*₂, small, loosely columnar head showing single series of sterigmata. *B* *A. effusus* NRRL No. 506. *B*₁, large radiate head showing double series of sterigmata. *B*₂, smaller head showing sterigmata in a single series. *B*₃, diminutive head borne upon one of a chain of foot cells. In this group single and double sterigmata often occur in the same head (not illustrated).

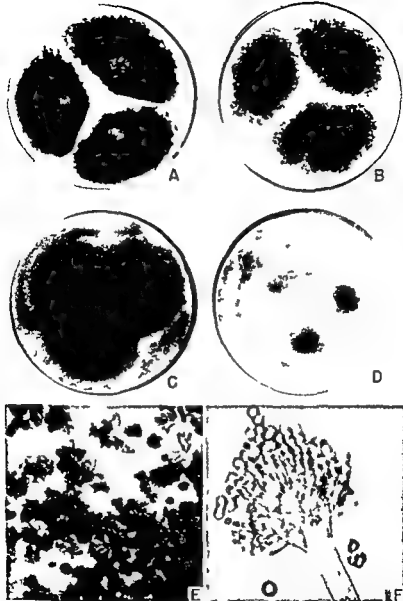


FIG 72 *Aspergillus flavus* A Typical strain NRRL No 1957 showing crowded conidial heads and occasional black sclerotia B Heavy sclerotium producing strain NRRL No 500 C Strain used for the production of kojic acid NRRL No 484 (This approaches the character of *A. oryzae*) D Dr White's strain of *A. flavus* (NRRL No 501) showing characteristic thin growth and sparse sporulation E Typical conidial heads $\times 18$ F Single head showing vesicle sterigmata conidia and the roughened conidiophore $\times 780$ All cultures on Czapek's solution agar 10 days

ter with walls pitted, rough (fig 72 F) almost spiny in appearance broadening upward and gradually enlarging into vesicles 10 to 30 or 40 μ in diameter, dome like in the smaller heads flask-shaped in larger heads (fig 72 F) Sterigmata in a single series in many smaller heads (fig 71 A₁) or both single and double series on the same vesicles in large heads (fig 71 A₁), varying from single sterigmata only 10 to 15 μ by 3 to 5 μ , to primary sterigmata 7 to 10 μ by 3 to 4 μ and a secondary series 7 to 10 μ by 2 \square to 3 μ Conidia pyriform to almost globose, nearly colorless to definitely yellowish green, varying from 3 μ , 3 by 4 μ , 4 by 5 μ , or even larger and marked variously with pits, echinulations, or irregularly winding color bars and ridges to give a roughened effect of varying intensity Sclerotia, when found, at first white then brown hard parenchymatous and a few strains white tipped produced by some strains regularly and abundantly (fig 72 B), scantily by others under undefined conditions Perithecia not found

Description originally based upon culture NRRL No 482 (Thom No 103) from the Centraalbureau at Baarn Holland but supplemented by observation of many hundreds of cultures from many substrata and all parts of the world

Unless segregation under a specific name is supported by adequate morphological and reproducible cultural data there is no way to identify the organisms intended Applying these criteria no reasons are seen for the use of the following specific designations

A. wehmeri Constantin and Lucet in Ann Sci Nat Bot (IX) 2 167 1905

A. variabilis Gasperini in Atti Soc Toscana Nat Sci Pisa Mon 8 fasc 2 376 1887

A. pseudo flavus Saito in Centralb Bakt etc 2 Abt 18 No 1/2 p 34 figs 15-18 1907 or its synonym *S. pseudo flava* (Saito) Sacc in Syll 22 1760 1913

A. siebenmanni Constantin and Lucet in Ann Sci Nat Bot (IX) 2 162 1905 is a bibliographic species based upon an organism isolated from the human ear and diagnosed by Siebenmann (Zeitsch f Ohrenheilk 12 1883) as *A. flavus* The describers regarded it as a separate species based only upon the description given by Siebenmann

A. gymnosardae Yukawa in Jour Col Imp Univ Tokyo 1 367 Pl 18 figs 1-7 1911 A member of the *A. flavus-oryzae* group with measurements intermediate between more typical representatives of the two species

A. thomii Graff nomen nudum a heavy sclerotium producing strain distributed by Graff but never described No diagnostic basis for the name was presented

A. pollinis Howard in Am Bee Jour 36 577-578 1896 was discussed as an organism causing pickled brood and bee paralysis (See also idem 38 530-531 1898) Turesson (Svensk Bot Tidskr 11 30 1917) decided the mold was *A. flavus*

Aspergillus parasiticus Speare in Hawaiian Sugar Planters Exp Sta

Path and Physiol Ser Bul 12 p 38 pl 3-4 1912 See

Thom and Church The Aspergilli, p 203 1926

Colonies on Czapek's solution agar with sucrose spreading rapidly forming a surface growth of crowded conidiophores with very few sterile

hyphae (fig 73 A) in deeper yellow green shades near ivy green (Ridgway Pl XXI) reverse uncolored or yellowish. Conidial heads radiate abundantly produced and giving color to the colony. Conidiophores given by Speare as 300 to 700 μ long commonly under 400 μ with walls colorless prominently rough or pitted enlarging from 3 μ at the foot up to 10 to 12 μ and passing into vesicles up to 35 μ in diameter (fig 73 B). Sterigmata in one series 7 to 9 μ by 2.5 to 3 μ closely packed over the vesicular surface yellow. Conidia pyriform to globose very rough 4 to 5 μ occasionally 6 μ in long axis green. No sclerotia or perithecia reported.

Described as parasitic upon the mealy bug of sugar cane (*Pseudococcus calceolariae* Mask.) in Hawaii. Type culture NRRL No 502 (Thom No 3509) received from Speare. Cultures with the same morphology were isolated from infected mealy bugs from Demerara by Thom and in Louisiana by Hopeloff. Johnston working in Puerto Rico considered that he had proved infectivity to be a strain function among organisms of the *A. flavus* series rather than associated with morphology. Blochwitz (Ann Mycol 32(1/2) 86 1934) has called another nearly allied form *A. flavus* var. *viridis* but gives no adequate data for separation. Cultures with these characters are occasionally obtained from sources not known to be associated with disease of insects. Shih has likewise described from China as *Aspergillus chungii* (Lingnan Sci Jour 15(3) 378 1933) a strain which apparently duplicates *A. parasiticus*.

Aspergillus effusus Tiraboschi in Ann di Bot (Rome) 7 16 fasc 1

1908. See also Thom and Church in Am Jour Bot 8 109-110

1921 and Thom and Church in The Aspergilli p 203 1926

Colonies on Czapek's solution agar rapidly and broadly spreading floccose or piled cottony white (fig 73 C) becoming dirty yellowish or in restricted areas pale greenish yellow then passing over into dull buff or tan shades as heads mature reverse and agar yellowish. Conidial heads usually more or less columnar mostly small a few of them fairly large many of them upon short conidiophores (fig 71 B₁ and 73 E) often less than 100 μ long and 5 to 10 μ in diameter arising from the trailing floccose hyphae quickly losing their yellow green color. Conidiophores with walls pitted or roughened sometimes bearing granules (produced by drying droplets of exuded fluid). Vesicles mostly under 20 μ in diameter (fig 71 B₂). Sterigmata in one series in small heads in either one or two series in large heads (fig 71 B₁) approximating the *A. flavus* type. Conidia pyriform to globose varying from 3 by 4 μ to 5 by 7 μ . No sclerotia or perithecia reported. The species was described originally from rotten corn (Zea Mays).

Culture description as given centers around culture NRRL No 506 (Thom No 130) isolated by Dr B F Lutman Burlington Vermont. Other

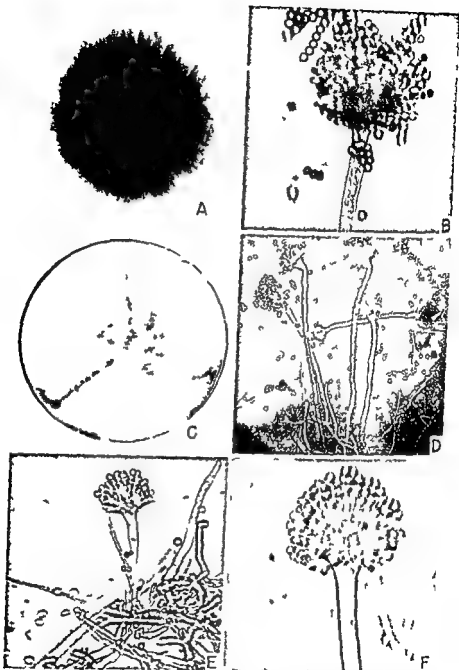


FIG. 7. A and B, *Aspergillus parasiticus* NRRL No. 465. A Colony on Czapek solution agar showing heavy production of short stalked conidial structures 10 days. B Single conidial head showing single series of sterigmata and roughened conidiophore $\times 500$. C-F *Aspergillus effusus* NRRL No. 506. C Colony on Czapek solution agar showing characteristic floccose habit and light sporulation 10 days. D Conidial structures arising from aerial hyphae characteristic of species $\times 165$. E Single short stalked fruiting structure arising from aerial hyphae $\times 300$. F Conidial head showing sterigmata mostly in two series, roughened surface of conidiophore not apparent $\times 500$.

strains studied include isolations from cornmeal from Indiana and from mealy bugs in Puerto Rico. Superficially this species bears little relation to *A. flavus*. Detailed microscopic examination of heads and spores however, reveals true and close relationships. Selective transfer from original colonies permitted changes in the predominance of the sterile areas over the fruiting areas without changing the general habit or nature of the colony. Blochwitz (Bot. Centralb. Beiheft Abt. Anat. Phys. 48: 176-182, 1931) regarded *A. effusus* as a floccose type of *A. flavus*. This position can be supported but the authors feel that the species should be maintained since it is strikingly different from *A. flavus* in its general colony appearance, and since it is repeatedly although infrequently isolated from nature.

Whether *Sterigmatocystis lutea* Baurer (Bul. Soc. Bot. France 27: 27, 1880) was one of these can only be guessed from culture. NRRL No. 508 (Thom No. 4640-473) received from the Baurer collection under this name. The strain is close to *A. effusus*. Baurer did not claim identity with *S. lutea* van Tieghem (Bul. Soc. Bot. France 24: 103, 1877) which was entirely undescribed.

Aspergillus jeanselmei Ota in Ann. de Parasit. 1(2): 137-146, 1923 as received from Baarn in 1939 (NRRL No. 507, Thom No. 5665) represented a member of the *A. flavus* series with close affinities to *A. effusus*.

Occurrence

Members of the *A. flavus-oryzae* group are among the most abundant of all the Aspergilli. They are world wide in distribution and are omnivorous in the substrata upon which they are able to grow and develop. They have been isolated from the widest variety of sources including the fermentation industries of the Orient, grains and cereal products from different parts of the United States, various types of forage, egg noodles, bread and other bakery products, leather goods, dried dates, cured meats, dairy products, nut meats, soy sauce, home-canned fruits and vegetables, textiles, paper, pulps, insects, tannin inoculum, feces, sputum, the lung of a bird, and from the duodenum of man. They are very abundant in soil and have been observed in almost all samples examined. They appear to be particularly common in the warm soils from tropical and sub-tropical areas. They vary greatly in cultural appearance and in the detailed measurements of their fruiting structures, and to a limited degree these differences can be correlated with the sources from which they are obtained. Soil isolates commonly show conidial heads near yellow-green in color which are borne upon comparatively short conidiophores. Isolates from the rice and soy fermentations of the Orient often show conidial heads pale yellow-green in color that are borne upon long, thin-walled conidiophores. Exceptions to this very general statement are common.

Kojic Acid

The ability of members of the *A. flavus oryzae* group to produce kojic acid has been recognized for more than three decades. It is only within the past fifteen years, however, that serious attention has been given to this fermentation. Beginning with the work of Challenger, Klein and Walker in 1929 and 1931, and continuing with that of May, Herrick, Moyer, Ward and Wells in 1931 and 1932, the proper nutrients and cultural conditions necessary for its production were defined. Subsequent contributions have been made by Kluyver and Perquin (1933) and by Barham and Smits (1936). In all of the early reports the responsible cultures were cited as *A. oryzae*, whereas in more recent ones the cultures employed have generally been identified as *Aspergillus flavus*. It is of interest to note that the strain studied by May and associates (NRRL No. 481, Thom No. 3538) was a thoroughly typical *A. flavus* when first isolated by Thom in 1914, but during the long period that it has been maintained in artificial culture it has gradually changed until today it more nearly resembles *A. oryzae* in its general habit and coloration (fig. 72 C). Its capacity to produce kojic acid remains undiminished, however. The culture employed by Barham (NRRL No. 625) likewise fails to satisfy the typical cultural picture of *A. flavus*, although it is discussed under this name. In contrast to these cultures, other strains belonging to this group have been under continuous laboratory cultivation for more than 30 years without apparent change in appearance or behavior. The above and additional references to the kojic acid fermentation are presented in the Topical Bibliography, pp. 297-298.

Enzymes

Members of the *A. flavus-oryzae* group produce diastatic and proteolytic enzymes abundantly. For this reason they have been much studied, and an extensive literature regarding mold enzymes has developed around the use of these fungi. In large measure the alcoholic and soy food industries of the Far East are based upon these molds and their enzymes. In the production of alcoholic beverages, the diastatic enzymes produced by an *Aspergillus* (regularly identified as *A. oryzae*) are employed to hydrolyze the rice starch. Alcohol is then produced from the resultant sugars by the addition of a fermentative yeast. In the soy industries, closely related molds, or even the same strains, are used as a source of proteolytic enzymes. In 1894 Takamine secured a series of U. S. patents covering the production of diastatic enzymes and the making of alcoholic liquors (see Topical Bibliography, p. 302). Subsequent to this, other investigators, mostly Japanese, published a number of papers in this field. Oshima in 1922 and 1928 reported on the production of protease by members of the *A. flavus*

oryzae group. Today considerable quantities of diastatic enzymes, proteolytic enzymes, and mixed diastatic and proteolytic preparations are being manufactured from these molds for use in the textile and tanning industries particularly. Within recent years considerable attention has been given to 'moldy bran' (bran seeded with selected strains of *A. oryzae*) as a possible substitute for malted barley as a saccharifying agent in the production of industrial alcohol (Underkofler, Fulmer and Schoene 1939; Schoene, Fulmer and Underkofler 1940; Hao 1942; Hao, Fulmer and Underkofler 1943; Christensen 1943).

References to papers dealing with the production of enzymes by molds belonging to this group are presented in the Topical Bibliography, pp. 302-304. No attempt has been made to present a complete bibliography of the subject, but it is believed that sufficient citations are listed to introduce the reader to the extensive literature of the field.

Pathogenesis (See Topical Bibliography, pp. 307-310)

Pathogenesis has been occasionally reported for strains identified as members of the *A. flavus* series. Observations reporting their presence in the external ear go back to Siebenmann (1882). Ota described *A. jeanselmei* as a parasite of human nails in Paris in 1923. Bereston and Keil (1941) described a case of infected nails in which the strain, as seen by us, proved to be a variant of *A. flavus*. There are ample records to show an occasional infection of the human being. There are no data as to the route of infection and the question whether the parasite is a primary or a secondary (wound) parasite stands unanswered. The constant presence of members of this group in every human environment together with a lack of evidence of ability to penetrate sound human tissue leaves some doubt—not as to its ability to grow when once established but whether it can actually break and enter as a direct agent of disease. *Aspergillus flavus* is commonly isolated from sputum. In birds, cases of lung involvement have been reported. There is no question but that the mold can persist for reasonable periods of time within the animal body. *A. flavus* is one of the more common air-borne molds and occasional allergic reactions are attributed to it although instances where it is the sole responsible agent are not known to have been reported.

Antibiosis

The production of antibacterial substances by strains of *Aspergillus flavus* has been observed by a number of workers during the past five years. White (1940) reported a culture of *A. flavus* (found by the writers to be a somewhat atypical strain) to produce some substance which was definitely bactericidal against some gram-negative and some gram-positive bacteria.

A more detailed study of this strain was subsequently made by White and Hill (1943) and the name "aspergillie acid" was assigned to the active substance. The compound shows a comparatively high toxicity to laboratory animals. Utilizing the White strain, Jones, Rake, and Hamre (1943) have made additional studies on the biological properties of aspergillie acid. In the meantime Glister (1941), at Oxford University reported the production of an antibacterial substance effective against gram negative and gram positive bacteria by a different strain of *A. flavus* (found by the writers to be wholly typical of the species). He noted the possibility of relationship between the substance with which he was working and that earlier reported by White.

Following the work of Jones, Rake, and Hamre (1943), McKee and MacPhillamy (1943) succeeded in demonstrating the production of a second and entirely different antibacterial substance. In certain chemical properties and in its action on bacteria this was found to resemble penicillin very closely, but actual identity was not proved. In a subsequent and more detailed report, McKee, Rake, and Houck (1944) defined more exactly its bactericidal action against various gram negative and gram positive bacteria and proved additional evidence of its penicillin like characters. They designated the substance "flavacidin".

Concurrent with this work Bush and Goth (1943a and 1943b), working at Vanderbilt University succeeded in demonstrating the production of an antibacterial substance from still another strain of *A. flavus* which was strongly active against *Staphylococcus* and other gram positive forms but comparatively inactive against gram negative forms belonging to the *E. coli* group. The substance was termed "flavacin". Identity with flavacidin and with penicillin is possible but has not yet been proved. Cook and Lacey (1944) report the production of appreciable amounts of an antibiotic substance from a strain of *A. parasiticus*. This was provisionally designated "parasiticin", and its similarity to penicillin was noted. Identity with flavacin (Bush and Goth) and flavacidin (McKee, Rake, and Houck) was suggested. Since *A. parasiticus* is so closely related to *A. flavus* it would seem probable that an antibiotic produced by it would be similar to that produced by the latter species under the same conditions.

Waksman and Bugie (1943) investigated a large number of strains belonging to the *A. flavus-oryzae* group. They found strains of *A. oryzae* to show little activity whereas strains of *A. flavus* showed increased but varying amounts. Yields were markedly influenced by various nutritional and environmental factors. Two types of antibacterial substances were observed: aspergillie acid and a substance similar to if not identical with penicillin. When grown in submerged culture one strain was found to produce amounts comparable to the best strains of *Penicillium notatum* tested. Unfortunately yields were not quantitatively determined.

CHAPTER XVI

THE ASPERGILLUS OCHRACEUS GROUP

Outstanding Characters

Conidial heads ranging from sulphur yellow to varying shades of ochraceous depending upon the species and strain showing a greenish tint only in the single species *A. sparsus* heads globose or radiate with conidial chains commonly adhering into divergent columns

Conidiophores normally showing shades of yellow in the outer layers of the wall which is rough or pitted usually prominently but occasionally reduced to traces which are seen most readily in dry mounts

Sterigmata in two series with the primary often quite large and septate

Conidia in some series thin walled and smooth in others showing definitely double walls more or less roughened or echinulate

Sclerotia present in most species and strains often dominating the cultures in others entirely lacking When present ranging in color from cream or buff through pink and orange shades to purplish vinaceous

Molds belonging to this group are common wherever organic matter is decomposing under natural conditions In spite of great variation in superficial appearance length of conidiophore size of heads intensity and shades of color and sclerotium production they fit together into a great natural group of related forms Extreme variants in the several series can be easily considered separate species and have been so described but collections of great numbers of such forms present so many gradations that identification by description becomes doubtful if not impossible Allocation to series centered upon some described species gives a practical method of grouping together closely related members of the great aggregate

The name *ochraceus* derived from the pigment ocher and attached to the most abundant series in the group is an old mycological usage which was more or less definitely followed in Saccardo's use of it for a plaque in his *Chromotaxia* Ridgway analyzed the colors more exactly dropped the term *ochraceus* but included a plaque near to it as *Pl. XV Col. 15 YO Ochraceous Buff* The strains of this group however mostly show colors closer to the yellower tints in Ridgway's Plate XXX column 19 It is important that relationship with the great group shall be quickly grasped whether exact identity with particular strains already known is claimed or not

Group characterization The colony appearance of this group of *Aspergilli* varies greatly with the presence or absence of sclerotia Some species

low globose heads. Sclerotia were not reported. In our experience however they are seen not infrequently in cultures which otherwise fit the description of *A. sulphureus* as presented. In the sub series, represented by *A. quercinus* (Bainier, Thom and Church) sclerotia are very abundant and heads comparatively few and scattered among the sclerotia. In addition to these two names which roughly designate sections or series of isolates as we find them every gradation between these extremes may be anticipated.

The species is fairly common in soils, on cereal grains and upon decaying vegetation.

Several species have been described as having conidial heads sulphur yellow but doubtfully separable from *A. sulphureus*.

S. ochroleuca Spegazzini in Myc. Argent. v. p. 434 in Anal. Mus. Nac. Buenos Aires Ser. 3 T. 13. 1911.

S. auricoma Gueguen in Bul. Soc. Myc. France 16: 171-187 figs 1-48. 1899. This appears to have been a member of the series with primary sterigmata proliferating abundantly to form little conidiophores and secondary heads thus giving the head the appearance of bearing yellow hair. Such proliferation occasionally occurs in many species but has not again been found in this group.

S. vitellina Rudley in Jour. Bot. (London) 34: 152, pl. 257, figs 14-16. 1896. is described as producing bundles or coremia composed of partially adherent conidiophores. The organism does not appear to have been cultivated and has not since been reported.

Aspergillus quercinus (Bainier) Thom and Church in The Aspergilli: p. 186. 1926.

Synonym *S. quercina* Bainier in Bull. Soc. Bot. France 28: 78. 1881.

See also Sartory. Etude biologique du *Sterigmatocystis quercina* Bainier in Bul. Soc. Myc. France 26: 349. 1910.

Colonies upon Czapek's solution agar spreading broadly characterized by the presence of an aerial white mycelium and the abundant production of sclerotia over the whole area (Pl. VII E and fig. 74 A) or in sectors or variously distributed the mass changing from yellow to orange yellow and finally to rufous or brick red shades with the ripening of the sclerotia reverse in shades of yellowish-orange. Conidial heads in yellow tints near sulphureus scattered among the sclerotia or occurring in long stalked groups in the dryer areas of the culture tube or plate mostly up to 200 μ in diameter but occasionally larger up to 300 or even 400 μ (fig. 74 C). Conidiophores with walls mostly pale yellow especially in the outer layer, pitted (fig. 74 D) occasionally with abundant granules about 2 μ in thickness varying from short and inconspicuous in crowded sclerotial areas to very long tufts in dryer areas up to 2 or even several millimeters by 10 to 20 μ . Vesicles colorless crushing easily 35 to 45 μ in diameter fertile over

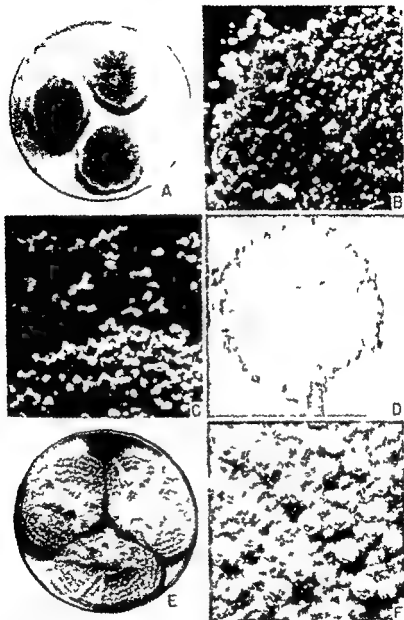


FIG. 74 A-D *Aspergillus quereus* NRRL No. 394 A Colonies on Czapek's solution agar 10 days predominantly a sclerotial but with localized areas of heavy conidial production in central areas B Marginal area of the same colony showing very abundant sclerotia and scattered conidial heads in lower right hand corner C Conidial heads on hay infusion agar plate $\times 18$ D Single head showing globose character crowded sterigmata and roughened conidiophore E *Aspergillus sclerorum* on Czapek's solution agar 10 days F Portion of the same enlarged to show abundant large sclerotia and scattered comparatively small heads $\times 6$

color and appearance (Pl VII F and fig 75 A) reverse ranges from colorless through orange to purplish shades

Conidial heads when large mostly globose or variously splitting into masses of conidial chains (fig 75 C) variously colored from very pale to deep ochraceous shades Conidiophores vary greatly in length and diameter

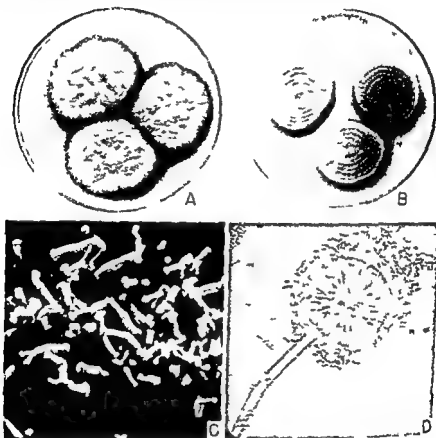


FIG 75 *Aspergillus ochraceus* series A and B 4 *ochraceus* NRRL No 398 and No 408 respectively, growing on Czapek's solution agar at room temperature 10 days C Mature heads of strain No 398 the tendency to split into divergent columns in age is characteristic D Photomicrograph of a single head showing globose vesicle sterigmata in two series and coarsely roughened conidiophore $\times 325$

in the various strains but typically show a yellow color in the outer layers of the thick wall which shows characteristic pitting or roughening (fig 75 D) Vesicles mostly globose or somewhat elliptical and fertile over the whole surface (fig 75 D) Sterigmata in two series primary varying from small to very large commonly 15 to 30 μ in length secondary fairly uniform

commonly 7 to 10 μ by 1.5 to 3.5 μ . Conidia globose subglobose or somewhat elliptical more or less rough or echinulate ranging from 3.5 to 5.0 μ in long axis. No perithecia reported. Sclerotia are present in Wilhelm's material preserved in exsiccatis.

Among the great number of isolates belonging to this group a considerable number show approximately the characters as drawn from Wilhelm's description and his exsiccatis. In the limited sense then *A. ochraceus* Wilhelm can be interpreted to apply to those ochraceous strains which bear echinulate conidia from 3.5 to 5.0 μ in diameter and produce sclerotia. Among the strains included almost all are found to produce colonies of consistent aspect in continuous culture.

Aspergillus elegans Ga. perini in Atti Soc. Toscana Sci. Nat. Pisa
Mem. 8 323 fasc. 2 1887

Synonym *S. elegans* (Gasp.) Sacc. in Syll. 10 525

This species by description differs little from *A. ochraceus* Wilhelm except for the absence of sclerotia and conidia which do not exceed 3.5 μ in diameter. Thom and Church did not recognize it as a valid species in 1926 and one may be certain that no sharp line can be drawn separating *A. elegans* from *A. ochraceus*. Nevertheless forms producing conidia consistently less than 3.5 μ are commonly encountered among miscellaneous isolations from nature and there is an argument for retaining a species to include such forms. The following species diagnosis taken from Saccardo (10 525) was presented by Thom and Church (1926).

Mycelium white, stalks continuous unbranched hyaline then pale ochraceous 1 to 6 mm long by 5 to 12 μ in diameter delicately studded with drops vesicle up to 70 μ diameter radiate entirely covered with sterigmata sterigmata primary 4 to 26 μ long secondary 7 to 14 μ long by 1 to 2 μ conidia ochraceous elliptical to globose up to 3 to 3.5 μ with wall very delicately verruculose sclerotia not found.

Various other species have been described which are obviously closely related and belong to the *A. ochraceus* series. A partial list would include

S. helva Bainier in Bull. Soc. Bot. France 28 78 1881. Thomas culture No 4640 476 received under this name came from the Bainier collection.

A. alutaceus Berkeley and Curtis (in Greville 3 No 2 p 108 1855) was the name proposed for a mold found upon corn. The specimen is preserved as No 3793 in Curtis Herbarium now in the Cryptogamic Herbarium of Harvard University. The specimen shows that this species was probably a strain of *A. ochraceus*.

A. ochraceus var. *microspora* Tiraboschi (in Ann. di Bot. [Rome] 7 14 1908) represents a strain in which all measurements were reported as reduced.

A. rehmsii Zukal. A culture from Dr. Westerdijk received under this name is also a member of this series.

Certain large-spored species of doubtful validity and relationship have been described that are believed to represent members of the *A. ochraceus* series. Although obviously rare, these species were described with sufficiently distinctive characters to warrant their retention. With continued search, it is possible that they may be reisolated.

Aspergillus delacroixii (Sacc) Thom and Church, in The
Aspergilli, p 190 1926

Synonym *S. ochracea* Delacroix in Bull Soc Myc France 7 109
Pl VII, fig f 1891 (Delacroix failed to recognize the
previous use of the specific name)
S. delacroixii Sacc in Sacc 10 527

This species is reported as having conidiophores pale yellow and rough 500 to 1000 μ in length, vesicle globose thick walled, punctate yellow, sterigmata in two series primary 39 μ by 12 μ secondary (from Delacroix's figures) about 8 to 10 μ by 2 to 3 μ conidia globose finely roughened 7 to 8 μ in diameter. The yellow and roughened conidiophores ally this species with the *A. ochraceus* group, hence would justify the description of a form with such large conidia should be found again. Otherwise it is possible that some old material of a strain of *A. oryzae* might have furnished the type.

Aspergillus butyracea (Bainier) n. comb.

Synonym *S. butyracea* Bainier in Bull Soc Bot France 27 20 1880
Specimen attributed to Bainier in C. Roumeguere's Fungi
Gallici Exsiccati No 995

This is described as a large spored strain belonging in this group. The specimen showed a black *Aspergillus* as well as an ochraceous form with spores up to about 6 μ and rough. Colonies butter yellow including conidiophores heads and conidia conidiophores yellow in mounts finely punctate or pitted 13 to 16 μ in diameter sterigmata primary up to 25 μ secondary 10 to 12 μ in length conidia described as smooth 5.2 μ but those found in the material were rough and up to 6.3 μ . It is entirely possible that this species may be found again but it has not been reported since Bainier described it.

A. penicillopsis (Hennings) Racib. P. Hennings Synonym *Stilbothamnium penicillopsis* P. Henn. and E. Nym. described in Fungi Monsunenses (Warburg O. Monsumia Bd I p 37 1900 Leipzig) exsiccati of type in Pathological Collections U. S. Dept. Agr. Bureau of Plant Industry as Raciborski no 87 in Crypt. Parasiticae Java.

This material shows an *Aspergillus* with the color and general appearance of *A. ochraceus* but of gigantic proportions. Measurements as follows

Conidiophore 50 to 70 μ in diameter 10 mm or more long with walls 7 to 12 μ thick Surface ragged and more or less pitted Vesicle up to 175 μ in diameter with walls 7 μ thick marked with a deep pit for each sterigma Sterigmata primary 50 to 90 μ at times 120 by 8 to 10 μ at the outer end sometimes with one cross wall secondary 15 to 25 μ by 3 to 4 μ clustered on the apex of the primary Occasionally with a sterile cell interposed between secondaries and primaries Conidia 8 to 12 μ by 5 to 8 μ elliptical pitted yellowish

Gigantic forms such as this occasionally appear under field conditions hence reach fungus herbaria Whether these are really different from some of the usual species can only be determined by collection and laboratory cultivation Until such study has been made such forms as *A. penicil* *lopsis* must be questioned

Aspergillus ostianus Wehmer in Bot Centralb 80 440-461 1899 also Monogr pps 117-119 Taf II No 1 1899-1901

Colonies upon Czapek's solution agar growing fairly well producing a surface growth of crowded conidiophores and conidial heads in yellowish to ochraceous shades passing to shades of cinnamon in very old cultures with reddish brown colors in reverse (Wehmer reported rusty yellow, pale to deep brownish yellow to cinnamon) Conidial heads globose up to 200 μ in diameter Conidiophores yellow Coarsely roughened mostly 500 to 700 μ long in crowded areas becoming 1 to 2 mm in margins of old colonies and usually 7 to 10 μ in diameter with walls heavy up to 2 or 2.5 μ in thickness Vesicles commonly globose about 40 μ in diameter or occasionally much larger up to 70 μ in growing heads thin walled colorless crushing easily leaving the funnel like yellow tip of the conidiophore open Sterigmata in two series primary from 15 to 20 μ long in smaller heads to 35 μ by about 8 μ at the tip in large heads secondary 10 to 13 μ by about 3 μ Conidia commonly 3 to 4 μ or even 5 μ in long axis varying from pyriform to elliptical or at times subglobose rough Sclerotia occasionally present but not conspicuous

This diagnosis was based upon Wehmer's description and is reasonably well represented by strain NRRL No 420 (Thom No 4724 35) received from Raistrick in 1924 and reported by him to have come from Westerdijk as Wehmer's original strain With conspicuous appearances suggesting relationship to the *A. ochraceus* group the shape and ultimate color of the ripe spores suggest a border line position between *A. ochraceus* and *A. tamarii*

Aspergillus sparsus Raper and Thom in Mycologia 38 572-574 fig 6 1944

Colonies upon Czapek's solution agar at room temperature spreading broadly dull grayish brown in color at first largely submerged but later

developing limited aerial growth, giving rise to widely scattered or conidial structures (fig 76 A) characterized by dull greenish tan heads affecting the color of the colony as a whole, never in brown shades or none. Colonies upon hay infusion agar spreading broadly, almost wholly submerged giving rise to scattered but conspicuous conidial structures (fig 76 B) often in definite concentric zones heads globose radiate, in olive

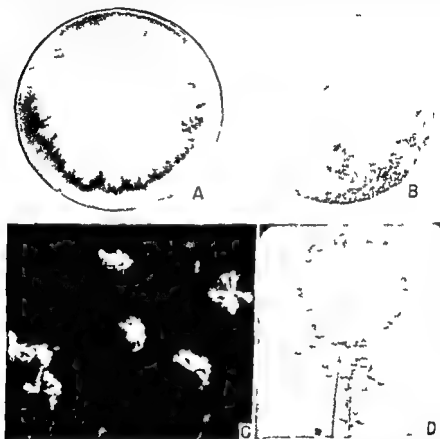


FIG 76 *Aspergillus sparsus* A and B Colonies on malt extract and hay infusion agars respectively 2 weeks note scattered heads in B and almost complete absence in A C Conidial heads $\times 18$ D Single conidial head showing globose vesicle and roughened conidiophore $\times 425$

buff shades (Ridgway, Pl XL) Colonies upon malt extract agar spreading irregularly floccose 1 to 2 mm deep cream buff in color conidial structures very few in number, heads globose radiate dull olive yellow in color (Ridgway, Pl XL) Conidial structures generally few in number never abundant erect arising from a submerged mycelium heads typically globose becoming more or less radiate in age (fig 76 C) mostly 200 to 250 μ

in diameter occasionally as much as 500μ pale olive buff to olive buff (Ridgway Pl XL) upon Czapek and hay infusion agars to pale olivine or olivine (Ridgway Pl XLII) upon malt extract agar. Conidiophores straight mostly 1 to $1\frac{1}{2}$ mm in length by 10 to 12μ in diameter approximately uniform in diameter throughout, wall 12 to 15μ in thickness conspicuously echinulate typically arising from a foot cell enmeshed in a network of feeder hyphae often tapering abruptly in the region immediately beneath the vesicle. Vesicle comparatively thin walled globose (fig 76 D) mostly 40 to 50μ in diameter occasionally larger or smaller bearing sterigmata over the entire surface. Sterigmata in two series primaries crowded comparatively short and stout commonly 8 to 10μ by 3 to 5μ secondaries 6 to 8μ by 2.5 to 3.5μ . Conidia pale yellowish in mass individually showing slight coloration subglobose to slightly elliptical very finely roughened mostly 3 to 3.5μ in long axis.

Type culture NRRL No 1933 was isolated in February 1943 from soil collected in La Lima Honduras by Dr L A Underkofler. A second strain which duplicates the type almost exactly was subsequently isolated from soil collected in Bixar County Texas by Sister Mary Clare of Our Lady of the Lake College San Antonio Texas.

The correct position of this species within the genus *Aspergillus* is open to question. The presence of a colored coarsely roughened conidiophore indicates close relationship with *Aspergillus ochraceus*. This is likewise supported by the globose vesicle and head although these characters are typical of other groups as well. The scarcity of fruiting structures upon all media and more particularly the greenish tint of the spore masses however tend to set it apart from the common representatives of this great group. The general habit of the colonies together with the paucity of conidial structures is strongly suggestive of *Aspergillus alliaceus* but this latter species does not show any trace of greenish color in its conidial heads it does possess smooth colorless conidiophores and upon ordinary culture media regularly produces an abundance of black sclerotia which very often dominates and characterizes the culture. In the color and character of its conidial heads *A. sparsus* is somewhat suggestive of George Smith's new species *A. alienaceus* (Trans Bul Mycol Soc 25 24-27 Pl I 1943) but it differs from this as it does from *A. alliaceus* in possessing rough conidiophores and in its failure to produce sclerotia. Until additional related forms are isolated we believe it best to consider this species as a member of the *A. ochraceus* group realizing that it does not entirely fit this placement as the group has hitherto been considered.

Occurrence and Economic Importance

Members of the *A. ochraceus* group are widely distributed in nature and can be obtained from a variety of sources. They are especially common

in soils and have been isolated from samples collected in many parts of the world. Within the group strains approximating the species *A. ochraceus* are by far the most abundant, although heavy sclerotium producing strains approximating *A. quercinus* are not infrequently encountered. They are a common component of the microflora of decaying vegetation, but there is little evidence that they play a very active role in processes of decomposition.

Huber (1933) found a member of the group *A. sclerotiorum*, capable of rotting apples and pears. Members of the group are commonly found in musty or moldy cereal grains, but are not as characteristic of this substratum as *Aspergillus candidus* and members of the *A. glaucus* group. In at least one instance *A. ochraceus* was reported as a human pathogen (Ceni 1905).

In the Orient, *A. ochraceus* and allied species constitute a portion of the mold flora characteristically found on "Katsuobushi" and other fermented preparations made from fish (Yukawa 1911). *Aspergillus melleus* Yukawa was isolated from such material. Because of the mixture of forms present, including members of the *A. glaucus* and *A. flavus oryzae* groups, it is probably incorrect to say that any particular species or group of species is responsible for this fermentation. (See also Hanzawa 1911).

A. ochraceus has been used to bring about desired changes in the flavor of coffee and its use is covered by U. S. Patent No. 1,313,209. Samples of the fermenting coffee showed the organism used to be a strain of *A. ochraceus* indistinguishable from Wilhelm's species. Whereas *A. niger*, *A. tamarii*, and *A. flavus* were also capable of developing in the fermenting coffee, *A. ochraceus* alone of the species tried gave a satisfactory flavor.

As a whole, the *A. ochraceus* group constitutes a very abundant but little studied, group of molds. Whether the scarcity of published reports regarding biochemical activities indicates an absence of such, or whether it merely reflects a limited amount of investigation, can at present only be guessed.

PART III

REFERENCE MATERIAL

CHAPTER XXII

TOPICAL BIBLIOGRAPHY

In preparing this manual the writers have considered it inadvisable to attempt to discuss the biochemical activities of the *Aspergilli* since this subject is book length in itself. Furthermore the aim of the manual is to provide the mycologist and microbiologist with a means of identifying and interpreting *Aspergilli* as they are isolated from nature and to furnish the chemist working with molds with a guide which will enable him to maintain industrially important cultures in an optimum condition. Nevertheless because of the increasing importance of the *Aspergilli* as agents responsible for industrial fermentations as subjects for physiological and biochemical investigations and now as possible sources of various antibiotics it has seemed advisable to present a topical bibliography dealing with these and related subjects. We have not attempted to present a complete bibliography but rather to present a sufficient number of references to provide the investigator and student with an entrée to the literature of particular fields. An attempt has been made to choose the more important papers for citation. Believing that more recent contributions will generally be of the greatest interest and value to the user this topical bibliography is presented in chronological rather than alphabetical order. A list of subjects under which references are presented follows.

CONTENTS OF TOPICAL BIBLIOGRAPHY

Acid Production by <i>Aspergilli</i>	290
Citric Acid	290
Fumaric Acid	293
Gallic Acid and Tannin Fermentation	294
General Papers and Reviews	294
Gluconic Acid	295
Itaconic Acid	297
Kojic Acid	297
Oxalic Acid	298
Miscellaneous Acids	299
Antibiotics and Toxins	300
Chemistry of Mold Tissue	301
Enzyme Production by <i>Aspergilli</i>	302
<i>Aspergillus flavus-oryzae</i> group	302
<i>Aspergillus niger</i> group	304
<i>Aspergilli</i> General	305
Fat Production by <i>Aspergilli</i>	306
Pathogenicity of the <i>Aspergilli</i>	307
Physiology of the <i>Aspergilli</i>	310

Pigments and Coloring Substances	312
Soil Tests for Mineral Deficiencies	313
Variation in the Aspergilli	314
Vitamins and Growth Substances	316
Miscellaneous Products	316
Alcohol	316
Chitin	317
Ergosterol	317
Fluorescein	317
Gums	317
Hydroxylamine	317
Mannitol	317
Polysaccharides	318
Ochracin	318
Terrein	318

ACID PRODUCTION

Citric Acid

- WEHMER C 1893 Note sur la fermentation citrique Bull Soc Chim France 9 728-730
- WEHMER C 1893 Préparation d'acide citrique de synthèse par la fermentation du glucose Compt Rend 117 332
- WEHMER C 1894 Process of making citric acid U S Patent 515 033 Feb 20
- WEHMER C 1897 Über Zwei weitere freie Citronensäure bildende Pilze Chem Ztg 21 1022-1023
- WEHMER C 1912 Über Citronensäuregärung Chem Ztg 36 1106-1107
- CURRIE J N 1917 The citric acid fermentation of *Aspergillus niger* Jour Biol Chem 31 15-37 pls 2 figs 5
- MOLLIARD M 1919 Production d'acide citrique par le *Sterigmatocystis nigra* Compt Rend Acad Sci (Paris) 168 360-363
- BUTKEWITSCH W 1923 Über die Citronensäuregärung Biochem Ztschr 142 195-211
- MOLLIARD M 1924 Influence de la nature des sucres sur la formation d'acides organiques par le *Sterigmatocystis nigra* en milieu déséquilibré Compt Rend Soc Biol (Paris) 90 1395-1397
- WEHMER C 1925 Bildung von citronensäure aus glykolsäure durch pilze Ber Deut Chem Gesell 58 2616-2619
- BERNHAEUER A 1926 Über die Säurebildung durch *Aspergillus niger* III Die Bedingungen der Zitronensäurebildung Biochem Zeitschr 172 324-49
- HENNEBERG W 1926 Handbuch der gärungsbakteriologie 2 v illus Berlin
- AMELUNG H 1927 Beiträge zur Säurebildung durch *Aspergillus niger* Zeit Physiol Chem 166 161
- CHALLENGER F SUBRAMANIAM V AND WALKER T A 1927 The mechanism of the formation of citric and oxalic acids from sugar by *Aspergillus niger* Jour Chem Soc (London) 200-208
- KOSTITSCHEV S AND TCHERNOKOV W 1927 Bildung von Zitronensäure und Oxalsäure durch *Aspergillus niger* Planta Abt E Zeits Wiss Biol 4 181-200

- WALKER TH K, SUBRAMANIAM V AND CHALLENGER F 1927 The mechanism of the formation of citric and oxalic acids from sugars by *Aspergillus niger* II Jour Chem Soc London 3044-54
- BERNHAEUER K 1928 Zum Chemismus der Citronensäurebildung durch Pilze I Die Säurebildung aus verschiedenen Kohlenstoffverbindungen Biochem Zeitschr 197 300-26
- BERNHAEUER K 1928 Zum Chemismus der Citronensäurebildung durch Pilze II Die Citronensäurebildung aus Gluconsäure Biochem Zeitschr 197 327-40
- BERNHAEUER K AND SCHÖN K 1928 Zum Chemismus der Citronensäurebildung Durch Pilze III Mitteilung Über die Hypothesen Citronensäurebildung und das Auftreten von Acetaldehyd in den Pilzkulturen Biochem Ztschr 202 164-179
- BERNHAEUER K 1928 Beiträge zur Enzymchemie der durch *Aspergillus niger* Bewirkten Säurebildungsvorgänge I Ztschr Physiol Chem 177 86-106
- BERNHAEUER K 1928 Über die Säurebildung bei *Aspergillus niger* IV Die Bedeutung der Myzelentwicklung für die Säurebildung Biochem Zeitschr 197 287-308
- FERNBACH A AND YUILL J L 1928 Improvement in and relating to the processes for the production of citric acid U S Pat 1 691 965 and 1 691 966
- FILOSPOV M E AND MALINOVSKII V E 1928 O limonno-kislom drozenii Nauchnaia Zapiski 6 235-239
- ESTES J 1928 Process for producing citric acid by means of fermentation Canad Pat 251 180 1925 and U S Pat 1 679 186
- CAHN F J 1929 Perfectionnements à la fabrication de l'acide citrique par fermentation Fr Pat 675 236 and 675 237
- CHALLENGER F 1929 The production of citric acid by fermentation process Ind Chem 5 181-184
- AMELUNG H 1930 Wachstum und Säurebildung von *Aspergillus niger* unter Wasser Chem Ztg 54 118
- CHREBACEK T AND TIUKOV D 1930 Biochemische Umbildungen der Essigsäure durch Schimmelpilze und über den Chemismus der Citronensäurebildung Biochem Zeit 229 343
- BERNHAEUER K, SIEBENSÜGER H AND TSCHINKEL H 1931 Zum Chemismus der Citronensäurebildung durch Pilze IV Über die Umwandlung der Zuckersäure Biochem Ztschr 230 466-474
- BERNHAEUER K AND SIEBENSÜGER H 1931 Zum Chemismus der Citronensäurebildung durch Pilze V Die Citronensäurebildung aus Essigsäure Biochem Zeit 240 232-44
- TAYLOR J N AND BOYD H G 1931 United States citric acid industry now self sufficient U S Dept Com Bur Foreign and Dom Com Com Rpt 4 227-228
- BERNHAEUER K 1932 Die oxydative Gärungen Verlag Julius Springer Berlin
- MAY D E AND HERRICK H T 1932 Production of organic acids from carbohydrates by fermentation U S Dept Agr Circular 216
- POBOES N 1932 Citric Acid Production by *Aspergillus niger* Am Jour Bot 19 559-567
- SAKAUCHI H AND YAMAYA J 1932 Production of citric acid by molds III Japanese black molds Jour Agr Chem Soc Japan 6 489-97
- KOSTITSCHEV S AND BERG V 1933 Conditions of Biochemical Production of Citric Acid Bull State Inst Agr Microbiol USSR 5 8-27

- BUTKEWITSCH W S MENZSCHINSKAYA E W AND TROFIMOVA E I 1934 Zur Biochemischen Herkunft von Citronen und Oxalsäure I Über die Bildung von Citronensäure aus Essigsäure Biochem Zeit 272 290 II Die Mycelsubstanzen als ein Quelle der Säurebildung Biochem Zeit 272 364
- DOELGER W P AND PRESCOTT S C 1934 Citric Acid Fermentation Ind Eng Chem 26 1142-49
- SOTNIKOV E 1934 Production of citric acid by *Aspergillus niger* I Production of citric acid in unchanged solutions Compt Rend Acad Sci USSR 3(4) 273
- SOTNIKOV E 1934 Production of citric acid by *Aspergillus niger* II Production of citric acid in changed solutions Compt Rend Acad Sci USSR 3(4) 279-83
- SOTNIKOV, E 1934 Production of citric acid by *Aspergillus niger* III Production of citric acid in often changed solutions Compt Rend Acad Sci USSR 3(7) 544-7
- ZUBKOVA GITLER S R AND ZALTS R M 1934 Preparation of Citric Acid from Wood Pulp Hydrolysates Lesokhimmicheskaya Prom 3 4-7
- BUTKEWITSCH V S AND GAWSKAYA, M S 1935 Yield of citric acid from sugar as a basis for estimating the mechanism of its formation from the latter Compt Rend Acad Sci USSR (N S) 3 405-408 Abstracted in Chem Abstr
- CARY F J 1935 Citric acid fermentation on solid materials Ind Eng and Chem 27 201-204
- CHRZASZCZ T AND PEYROS E 1935 Optimale Bedingungen der Citronensäureanreicherung sowie einiger Beobachtung zu Theorie der Citronensäurebildung Biochem Ztschr 280 325-336
- GIORDANI M 1935 Fermentazione citrica La Chimica e l'Industria 17 77-81
- GUDLET M A 1935 Gasregime of *Aspergillus niger* as related to citric acid formation Proc Inst Sci Res Food Ind (Leningrad) 3 No 1 45-69 Abstracted in Chem Abstr
- IVANOV N N 1935 Biochemical production of citric acid Proc Int Sci Research Ind (Leningrad) 3 No 1 3-4 Practical and theoretical studies
- KRESLING E K 1935 Effect of radium on citric acid formation by *A. niger* Proc Inst Sci Res Food Ind (Leningrad) 3(4/5) 130-145 No marked effect
- BERNHAEUER K AND ISLAUER A 1936 Über die Säurebildung aus Zucker durch *Aspergillus niger* VI Faktoren der Citronensäureanreicherung Biochem Ztschr 286 45-69
- CARY F J 1936 Citric Acid Manufacture U S Patent 2 047 669 July 14
- CHRZASZCZ T AND M ZAKOMORNY 1936 Über die Bedeutung der Apfel säure bei der Umbildung der Desigsäure in Citronensäure durch verschiedene Schimmelpilze Biochem Zeit 286 348-355
- CLITTERBUCK P W 1936 Recent developments in the biochemistry of moulds Jour Soc Chem Ind 14 55T-61T
- IVANOV N N 1936 Biochemical production of citric acid Proc Int Sci Research Ind (Leningrad) 3 No 4 5 Laboratory and semiplant stages to commercial production
- LAMB A R 1936 Utilization of (Cane) Molasses Proc Hawaiian Sugar Planters Assn 56th meeting 42-3
- WELLS P A MOYER A J AND MAY U E 1936 The chemistry of the citric acid fermentation I The carbon balance Jour Amer Chem Soc 55 555-558

- NAKAZAWA R TAKEDA Y AND NAKANO M 1937 Citric Acid Manufacture by Fermentation I *Aspergillus awamori* var *fumeus* Jour Agr Chem Soc Japan 11 57-62
- L VOY E AND TOUPRIZINA G M 1938 Effect of sodium fluoride on the formation of citric and gluconic acids by the fungus *Aspergillus niger* Compt Rend Acad Sci (USSR) 11 307-11
- WELLS P A AND HERRICK H T 1938 Citric Acid Industry Ind and Eng Chem 30 235-267
- ZENDER J 1938 Production of Citric Acid U S Patent 2 171 063 June 21
- BERNHAUER K 1939 Gärungschemisches Praktikum Zweite Auflage pp 317 Verlag Julius Springer Berlin
- ZHURAVKII G I 1939 The Gas Exchange in *Aspergillus niger* during the Formation of Citric Acid Microbiology 8 414-430 (USSR)
- BERNHAUER K KNOBLOCH H AND ZIFFELIUS O 1940 Über die Säurebildung aus Zucker durch *Aspergillus niger* VIII Der Einfluss von Magnesium auf die Säurebildung Biochem Ztschr 303 300-307
- DAS GUPTA G C SAHA K C AND GUHA B C 1940 The fermentative production of citric acid and oxalic acid from molasses J Indian Chem Soc Ind and News Ed 3 64-74
- MELNIKOVA A A AND TROFIMOVA E I 1940 Activation of the process of citric acid accumulation by surface molds of *Aspergillus niger* Microbiology (USSR) 9 555-68
- PRESCOTT S C AND DUNN C D 1940 Industrial Microbiology Chapt XXV The Citric Acid Fermentation pp 358-379 McGraw Hill N Y
- WANG Y 1940 Citric acid metabolism of *Aspergillus carbonarius* Jour Shanghai Sci Inst Sect IV 5 61
- BARINOVA S A 1941 Considerations in the appraisal of the circulating medium system of obtaining citric acid from cultures of *Aspergillus niger* Microbiology 10 854 (USSR)
- BERNHAUER K ISLAUER A AND KNOBLOCH H 1941 Über die Säurebildung aus Zucker durch *Aspergillus niger* X Der Einfluss der Zuckersorte auf die Citronensäurebildung Biochem Zeit 307 298
- KAROW EDWARD OTTO 1947 The Production of Citric Acid in Submerged Culture Doctoral Thesis submitted to Faculty of Rutgers Univ New Brunswick N J March 1947 Strain of *Aspergillus wentii* found to give good yields
- SEITZ JOSEPH 1941 Method of producing citric acid by fermentation U S Patent 2 353 771 July 18

Fumaric Acid

- ENRICH F 1911 Über die Bildung von Fumarsäure durch Schimmelpilze : Ber Deut Chem Ges 44 3737-3747
- WEHMER C 1918 Über Fumarsäure-Gärung des Zuckers Ber Deut Chem Gesell 61 1663 1668 figs 6
- ENRICH F 1919 Über Fumarsäure Gärung des Zuckers Ber Deut Chem Ges 52 63-64
- SCHREYER R 1928 Säuerungsversuche mit dem Pilz *Aspergillus fumigatus* Biochem Zeit 207 131 56
- THIES W 1930 Untersuchungen über den einfluss der bedingungen auf die säurebildung des schimmelpilzes *Aspergillus fumigatus* Centralb Bakt (etc) 82(2) 321-347

- THIES W 1931 Decomposition of salts of organic acids by the mold fungus *Aspergillus fumigatus* Ber 64B 214-8
- CHRZASZCZ T AND ZAKORONORNY M 1933 Biochemical Transformation of Sugar by Fungi The Transformation of Fumaric Acid the Accumulation of Formic Acid and the Chemistry of Oxalic Acid Formation Biochem Zeit 259 156

Galic Acid and Tannin Fermentation

- SCHÉELE A 1787 Über das wesentliche gallapfelsalz Crell's Chem Ann 1 3-12
- ROBIQUET E 1852 Recherches sur la fermentation gallique Jour Pharm 22 129-130
- LAROCQUE 1852 Remarques à l'occasion d'une communication récente de M. Fd Robiquet sur la fermentation gallique Compt Rend Acad Sci (Paris) 35 221
- VAN TIEGHEM PH 1867 Sur la fermentation gallique Compt Rend Acad Sci Paris 65 1091-1094
- DICLAUX E 1883 Chimie biologique In Frémy E Encyclopédie Chimique 9(1) 908
- FERNBACH A 1900 Sur la tannase Compt Rend Acad Sci Paris 131 1214-1215
- POTTEVIN H 1900 La tannase diastase dédoublant l'acide gallotanannique Compt Rend Acad Sci Paris 131 1215-1217
- CALMETTE A 1907 Verfahren zur Umwandlung von Tannin in Gallusäure Ger Pat 129 164
- KNUDSON L 1913 Tannic acid fermentation I Jour Biol Chem 44 159-184 illus
- KNUDSON L 1913 Tannic acid fermentation II Effect of nutrition on the production of the enzyme tannase Jour Biol Chem 44 185-202
- DALVI P D 1931 Biochemistry of tan liquor fermentation Jour Ind Inst Sci 13A 173-92
- NICHOLSON W N AND NIERENSTEIN M 1931 Action of tannase on gallotannin Biochem Jour 26 752-5
- DEYS W B AND HUKMAN M J 1937 Splitting off of gallic acid from tannin especially from theotannin by *Aspergillus niger* Proc Acad Sci Amsterdam 40 818-25
- CHI LAING HLO 1939 Researches on Fermentation for the Production of Gallic Acid III Effect of adding yeast Whang Hai 1(2) 11-19 cited C A 34 2129 1940
- KWANG CHUR HSIEN 1939 Researches on Fermentation for the Production of Gallic Acid IV Optimum concentration of gallnut extract and rate of fermentation Whang Hai 1(3) 1-3 cited C A 34 2129 1940
- WEN TER WEI 1939 Researches on Fermentation for the Production of Gallic Acid V Changes in tannin content gallic acid content and total activity in the fermentation liquor Whang Hai 1(3) 4-5 cited C A 34 2130 1940

General—Acid Production

- ELRYING F 1918-19 Über die Bildung organischer Säuren durch *Aspergillus niger* Öfvers Finska Vetensk Soc Förhandl 61 Afd A No 2 Art 15 pp 1-23

- FALCK R AND VAN BEYMA THOE KINGMA 1974 Methodisches und Prinzipielles zur Darstellung organischer Säuren auf biologischen Wege mit Hilfe von Fadenpilzen Ber Deut Chem Gesell 57 915-920
- CHALLENGER F SUBRAMANIAM V AND WALKER T K 1927 Formation of organic acids from sugars by *Aspergillus niger* Nature (London) 119 674
- HERRICK H T AND MAY O E 1929 Molds and Chemical Manufacture Ind Eng Chem 21 618
- MAY O F AND HERRICK H T 1930 Some minor industrial fermentations Ind Eng Chem 22 117
- MAY O E AND HERRICK H T 1932 Production of organic acids from carbohydrates by fermentation U S Dept Agr Bul No 216 1-30
- SAKAGUCHI K 1932 Acid and alcohol production by *Aspergillus* VIII The species of *Aspergillus* and the production of acids Jour Agr Chem Soc Japan 8 431-44
- MAY O E AND HERRICK H T 1934 Some practical and theoretical aspects of mold metabolism Jour Bact 28 145-151
- BERNHAEUER K 1936 Gärungschemisches Praktikum 249 pp Verlag Julius Springer Berlin
- BERNHAEUER K 1939 Gärungschemisches Praktikum 317 pp Verlag Julius Springer Berlin
- WELLS P A AND WARD G E 1939 Fermentation Processes Ind Eng Chem 31 172 177

Gluconic Acid

- MOLLIARD M 1922 Sur une nouvelle fermentation acide produite par le *Sterig malocystis nigra* Compt Rend Acad Sci Paris 174 881-883
- BUTKEWITSCH W 1924 Über die Bildung der Glucon und Citronensäure in den Pilzkulturen auf Zucker Biochem Zeitschr 164 177
- MOLLIARD M 1924 Nouvelles Recherches sur la formation d'acides organiques par le *Sterigmatalocystis nigra* en milieu déséquilibrés Compt Rend Acad Sci (Paris) 178 41-45
- BERNHAEUER K 1926 Über die Säurebildung durch *Aspergillus niger* I Allgemeine und Methodisches bei der Untersuchung der Säurebildungsvorgänge Biochem Zeitschr 172 296-312
- BERNHAEUER K 1926 Über die Säurebildung durch *Aspergillus niger* II Die Bildung der Gluconsäure Biochem Zeitschr 172 313-23
- BERNHAEUER K AND WOLF H 1928 Zur Enzymchemie der *Aspergillus niger* bewirkten Säurebildungsvorgänge II Zeits Physiol Chem 177 270-79
- MILLER D 1928 Studien über ein neues Enzym Glykoseoxydase I Biochem Zeits 199 136-170
- WEHNER C 1928 Abnahme des Säuregärungsvermögens und Änderung der Säure bei einem Pilz (Gluconsäure statt Fumarsäure-Gärung) Biochem Zeits 197 416-437
- MAY O E HERRICK H T MOYER A J AND HELLBACH R 1929 Semiplant scale production of gluconic acid by mold fermentation Ind Eng Chem 21 1198
- MÜLLER D 1929 Studien über ein neues Enzym Glykoseoxydase II Biochem Zeits 205 111-143
- HARRISON D C 1931 Glucose dehydrogenase a new oxidizing enzyme from animal tissues Biochem Jour 25 1016-1027

- SCHREYER R 1931 Comparative studies on the formation of gluconic acid by molds Biochem Zeit 240 295-325 *A. wentii* and *A. luchuensis*
- SAKAGUCHI A 1932 The production of acids and alcohol by *Aspergillus* IV. The production of d gluconic acid by *Aspergillus oryzae* Jour Agr Chem Soc Japan 8 264-265
- ANGELETTI A AND PONTE D 1933 Azione de alcuni funghi su soluzioni di aldosi e di altre materie zuccherine. Nota VI Formazione di acido ossalico dall'acido d gluconico Ann Chim Appl 25 33-37
- CURRIE J N KANE J H AND FINLAY A 1933 Production of gluconic acid by fungi U S Pat 1 893 819
- KARDO-SYSSOJEVA E 1933 The formation of gluconic acid by *Aspergillus niger* Biochem Zeit 266 337-351
- MAY O E HERRICK H T MOYER A J AND WELLS P A 1934 Gluconic acid production by submerged mold growth under increased air pressure Ind Eng Chem 26 575-578
- BOLCATO V 1935 La reazione del mezzo e l'attività dei feltri comuni e preformati di un *Aspergillus* Ann Chim Appl 25 423-432
- HERRICK H T HELLBACH R AND MAY O E 1935 Apparatus for the application of submerged mold fermentations under pressure Ind Eng Chem 27 681-683
- KARDO-SYSSOJEVA E 1935 Biochemical Production of Calcium Gluconate Prod Inst Sci Res 3 83-94
- MOYER A J WELLS P A STUBBS J J HERRICK H T AND MAY O E 1937 Gluconic acid production Development of inoculum and composition of fermentation solution for gluconic acid production by submerged mold growths under increased air pressure Ind Eng Chem 29 777-781
- WELLS P A LYNCH D F J HERRICK H T AND MAY O E 1937 Translating mold fermentation research to pilot plant operation Chem Met Eng 44 188-190
- WELLS P A MOYER A J STUBBS J J HERRICK H T AND MAY O E 1937 Gluconic acid production Effect of pressure air flow and agitation on gluconic acid production by submerged mold growths Ind Eng Chem 29 653-656
- GASTROCK E A FORGES N WELLS P A AND MOYER A J 1938 Gluconic acid production on pilot plant scale effect of variables on production by submerged mold growth Ind Eng Chem 30 780-789
- LYOFF S AND LOUFIZINA G M 1938 Effect of sodium fluoride on the formation of citric and gluconic acid by the fungus *Aspergillus niger* Compt Rend (Doklady) Acad Sci 21 307-311
- MOYER A J, UMBERGER, E J AND STUBBS J J 1940 Fermentation of Concentrated Solutions of Glucose to Gluconic Acid Improved Process Ind Eng Chem 32 1379-1383
- FORGES N CLARK T F AND GASTROCK E A 1940 Gluconic Acid Production Repeated use of Submerged *A. niger* for Semicontinuous Production Ind Eng Chem 32 107-111
- KNOBLOCH H AND MAYER H 1941 Über die Bildung von d Glucon d Mannon und d Galakturonsäure durch *Aspergillus niger* in Schüttelkultur Biochem Zeit 307 285
- FORGES N CLARK T F AND ARONOVSKY S I 1941 Gluconic Acid Production Repeated Recovery and Re-use of Submerged *Aspergillus niger* by Filtration Ind Eng Chem 33 1065-1067

- CARRERO JAIME GONZALEZ 1947 Obtaining Gluconic Acid by the Fermentation Method Ion 2 819 Abstracted in Chem Abst 37 6816 1943

Itaconic Acid

- KINOSHITA K. 1929 Formation of itaconic acid and mannitol by a new filamentous fungus Jour Chem Soc Japan 50 593-93
- KINOSHITA K. 1931 Über die produktion von itaconsaure und mannit durch einen neuen Schimmelpilz *Aspergillus itaconicus* Acta Ithytochimica 11 271-287
- KINOSHITA K. 1937 : Physiological and biochemical studies of *Aspergillus itaconicus* Acta Phytochim (Japan) 9 159-87
- CALAM C T OXFORD A E AND RAISTRICK H 1939 The biochemistry of micro-organisms LXIII Itaconic acid a metabolic product of a strain of *Aspergillus terreus* Thom Biochem Jour 33 1488-95
- LOCKWOOD L B RAPER KENNETH B MOYER A J AND COGHILL R D The production and characterisation of ultraviolet induced mutations in *Aspergillus terreus* III Biochemical characteristics of the mutations Am Jour Bot (in press)
- LOCKWOOD L B AND REEVES MAX D In press Some factors affecting the production of itaconic acid by *Aspergillus terreus*
- LOCKWOOD L B AND WARD G E In press Fermentation process for itaconic acid Jour Ind Eng Chem
- MOYER A J AND COGHILL R D In press The laboratory-scale production of itaconic acid by *Aspergillus terreus*
- RAPER KENNETH B COGHILL R D AND HOLLAENDER ALEXANDER The production and characterisation of ultraviolet induced mutations in *Aspergillus terreus* II Cultural and morphological characteristics of the mutations Am Jour Bot (in press)

Kojic Acid

- SAITO K. 1907 Über die Säurebildung bei *Aspergillus oryzae* Bot Mag (Tokyo) 21 7-11
- YABUTA T 1912 On Koji acid a new organic acid formed by *Aspergillus oryzae* Jour Coll Agric Tokyo 5 51-58 The paragraphs on koji acid were contributed by Dr J F Brewster
- YABUTA T 1924 The constitution of kojic acid a γ pyrone derivative formed by *Aspergillus oryzae* from carbohydrates Jour Chem Soc (London) 125 575-587
- TAMITA H 1927 Metabolism of *Aspergillus oryzae* I Acta Phytochim 3 51-173
- CHALLENGER F KLEIN L AND WALKER T K 1929 Production of Kojic Acid from Pentose by *A. oryzae* Chemistry and Indus 48 607
- KATAGIRI H AND KITAHARA K. 1929 The formation of kojic acid by *Aspergillus oryzae* Part I The formation of kojic acid from pentoses sugar alcohols and gluconic acid Bull Agr Chem Soc of Japan 5 111
- BIRKINSHAW J H CHARLES J H V LILLY C H AND RAISTRICK H 1931 Studies in the biochemistry of micro-organisms VII Kojic acid (5-hydroxy 1-hydroxymethyl- γ pyrone) Phil Trans Roy Soc London 220B 177-138
- CHALLENGER F KLEIN L AND WALKER T K 1931 The formation of Kojic Acid from sugars by *Aspergillus oryzae* Jour Chem Soc pp 16-23

- MAY O E MOYER A J WELLS P A AND HERRICK H T 1931 The production of Kojic Acid by *Aspergillus flavus* Jour Am Chem Soc 53 774-780
illus
- MAY O E WARD G E AND HERRICK H T 1932 The effect of organic stimulants upon the production of kojic acid by *Aspergillus flavus* Zentralbl Bakt Parasitenk u Infektionsk II Abt 86 129-131
- DI CAPUA A 1933 Action of iron on the formation of kojic acid by means of *Aspergillus flavus* Gazz Chim Ital 63 296-302
- KATAGIRI HIDEO AND KITAHARA KAKUO 1933 Formation of kojic acid by *Aspergillus oryzae* Mem Coll Agr Kyoto Imp Univ No 36 p 1-9
- KLUYVER A J AND PERQUIN L H C 1933 Conditions for the formation of kojic acid by *Aspergillus flavus* Link Biochem Zeit 266 87-90
- BARHAM H N AND SMITS B L 1934 Kojic acid—A review Trans Kansas Acad Sci 37 91-113
- FRIEDEMANN T E 1934 Chemical and physiological properties of kojic acid Science 80 34
- BARHAM H N AND SMITS B L 1936 Production of kojic acid from xylose by *Aspergillus flavus* Ind Eng Chem 28 567-70
- GOULD B S 1933 The metabolism of *Aspergillus tamarii* Kutz Kojic acid production Biochem Jour 32 797-802
- RAYMAN M M 1941 Production of chemicals by the fermentation of the acid hydrolyzate of oat hulls Ia St Coll Jour Sci 16 122-124 Kojic acid from oat hulls by *Aspergillus flavus*

Oxalic Acid

- DUCLAUT E 1889 Sur la nutrition intracellulaire Ann Inst Pasteur 3 97-112 illus
- WEHMER C 1891 Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze Bot Ztg 49 233-246 and others
- WEHMER C 1891 Zur zersetzung der oxalsäure durch licht und stoffwechselwirkung Ber Deut Bot Gesell 9 218-229
- WEHMER C 1892 Über oxalsäure-bildung durch pilze Liebigs Ann Chem 269 383-389
- WEHMER C 1897 Kleinere mykologische mitteilungen Zur Oxalsäuregärung durch *Aspergillus niger* Centralbl Bakt (etc) 2 Abt 3 102-103 147 153
- EMMERLING O 1903 Oxalsäurebildung durch Schimmelpilze Centralbl Bakt etc 2 Abt 10 273
- CURRIE J N AND THOM C 1915 An oxalic acid producing *Penicillium* Jour Biol Chem 22 287-293
- RAISTRICK H AND CLARK A B 1919 On the mechanism of oxalic acid formation by *Aspergillus niger* Biochem Jour 13 329-344
- WALKER T K SUBRAMANIAM V AND CHALLENGER F 1927 The mechanism of the formation of citric and oxalic acids from sugar by *Aspergillus niger* II Jour Chem Soc (London) 13044-3054
- CHRASCI T AND TILKOW D 1930 Oxalsäure in Schimmelpilsekulturen Biochem Ztschr 218 73-85
- HOFMANN EDWARD 1931 Formation of Oxalic Acid by *Aspergillus niger* from Uronic Acid Biochem Zeit 243 423-8
- BERNHAEUER K AND SLANINA F 1933 The chemistry of the processes of acid formation induced by *Aspergillus niger* V The formation of oxalic acid from formic acid Biochem Zeit 264 109

- WEBBER H A 1931 Production of Oxalic Acid from Cellulose Agricultural Material Ia Eng Expt Sta Bull 118 pp 55
- HORMANN EDWARD 1936 Acidification of the Culture Media by *Aspergillus niger* and the Formation of Oxalic Acid Arch Phys Biol 13 84-87
- ALLSOPP A 1937 Formation of oxalic acid by *Aspergillus niger* New Phytol 36 337-356
- JAQLOT R 1938 Oxalic Acid Fermentation Mechanism of Oxalicogenesis by Moulds Ann des Ferm 4 284-294
- JAQLOT R 1938 Influence of pH on the Production of Oxalic Acid by Various Molds Compt Rend Soc Biol 127 1431-1432
- JAQLOT R 1938 Production of Oxalic Acid by Molds Energy Expenditure Compt Rend Soc Biol 128 69-70
- MELNIKOVA A A AND BUTKEVICH V S 1939 Biochemical formation of oxalic acid from sugar Microbiology (USSR) 8 818-76
- SIMO MITLO 1939 Crystals occurring in the mold membrane in oxalic acid fermentation Jour Agr Chem Soc Japan 11 751-2

Miscellaneous Acids

- BERNHALER H BOCKI V AND EICHENAUER H 1932 The formation of acid from sugar by *Aspergillus niger* V The formation of malic acid besides citric acid Biochem Zeit 253 37
- BERNHALER H AND SCHELER Z 1932 The chemistry of the processes of acid formation induced by *Aspergillus niger* VI The formation of glycolic and glyoxylic acids from acetate salts Biochem Zeit 253 11
- BERNHALER H AND BOCKI V 1932 The chemistry of the processes of acid formation induced by *Aspergillus niger* VIII The transformation of acetic acid into citric acid and further notes on the degradation of acetic acid Biochem Zeit 253 25
- SAKAGUCHI H 1932 The production of acids and alcohol by *Aspergillus* VI The production of D gluconic acid by *Aspergillus oryzae* Jour Agr Chem Soc Japan 8 764-5
- KARDO-SUTSOEVA M 1933 Formation of glucuronic acid by *Aspergillus niger* Biochem Zeit 268 337-51
- NISHIKAWA H 1933 Biochemistry of the filamentous fungi II A metabolic product of *Aspergillus melleus* Yukawa Bull Agr Chem Soc Japan 9 107-109 Mellein and melleic acid
- NISHIKAWA H 1933 Biochemistry of the filamentous fungi III A metabolic product of *Aspergillus melleus* Yukawa Bull Agr Chem Soc Japan 9 148-151 Mellein and melleic acid
- SUMIKI Y 1933 Fermentation products of molds X *Aspergillus glaucus* Jour Agr Chem Soc Japan 9 714-16 Records formation of glaucic acid
- GLIMM E AND NITTSCHKE M 1934 Formation of malic acid from asparagine by fermentation at different pH values Biochem Zeit 268 444-50 A niger and yeast employed
- BIRKINSHAW J H 1937 Biochemistry of the lower fungi Biol Rev Cambridge Phil Soc 12 357 Records production of aconitic acid by *Aspergillus stactosus*
- STEINBERG R A 1942 The process of amino acid formation from sugars in *Aspergillus niger* Jour Agr Res 64 615-33

ANTIBIOTICS AND TOXINS

- BODIN E AND GAUTIER L 1906 Note sur une toxine produite par l *Aspergillus fumigatus* Ann Inst Pasteur 20 209-224
- BOAS F 1919 Auto-toxicity in *Aspergillus niger* Ber Deut Bot Gesell 37(1) 63-65
- WALKER J C LINDEGREN C C AND BACHMANN F M 1925 Further studies on the toxicity of juice extracted from succulent onion scales Jour Agr Res 30 175-187
- ANSLOW WINSTON K AND RAISTRICK HAROLD 1935a The biochemistry of micro-organisms LVII Fumigatin (3 hydroxy-4 methoxy 2 5-toluquinone) and spinulosin (3 6-dihydroxy-4-methoxy 2 5-toluquinone) metabolic products respectively of *Aspergillus fumigatus* Fresenius and *Penicillium spinulosum* Thom Biochem Jour 31 687-96
- ANSLOW WINSTON K AND RAISTRICK HAROLD 1935b The biochemistry of micro-organisms LIX Spinulosin (3 6-dihydroxy-4 methoxy 2 5-toluquinone) a metabolic product of a strain of *Aspergillus fumigatus* Fresenius Biochem Jour 31 2288-9
- WOOLLY D W BERGER J PETERSON W H AND STEENBOCK H 1938 Toxicity of *Aspergillus sydowii* and its correction Jour Nutrition 18 465-76
- WHITE EDWIN C 1940 Bactericidal filtrates from a mold culture Science 91 127 *Aspergillus flavus*
- GLISTER G A 1941 A new antibacterial agent produced by a mold Nature 148 470 *Aspergillus flavus*
- OXFORD A E AND RAISTRICK H 1942 Anti bacterial substances from moulds IV Spinulosin and fumigatin metabolic products of *Penicillium spinulosum* Thom and *Aspergillus fumigatus* Fresenius Chem and Ind 31 128
- TIMONIN M I 1940 Another mould with anti bacterial ability Science 96 No 2500 p 494 *Aspergillus* sp white spored
- WAKSMAN, SELMAN A HORVING ELIZABETH S AND SPENCER ERVEST L 1940 The production of two antibacterial substances fumigacin and clavacin Science 96 202 August 28
- WAKSMAN S A HORVING ELIZABETH S AND SPENCER ERVEST I 1940 Two antagonistic fungi *Aspergillus fumigatus* and *Aspergillus clavatus* and their antibiotic substances Jour of Bact 45 233-249
- WHITE E C AND HILL JUSTINA H 1942 Antibacterial filtrates from a strain of *Aspergillus flavus* Jour Bact 43 12
- WIESNER H P 1942 Bactericidal effects of *Aspergillus clavatus* Nature 149 3-6
- WILKINS W H AND HARRIS G C M 1942 Investigations into the production of bacteriostatic substances by fungi I Preliminary examination of 100 fungal species Brit Jour Exper Path 23 168
- BUSH M T AND GOTH A 1943 An antibacterial substance produced by an *Aspergillus flavus* Federation Proc 2 75
- BUSH MILTON T AND GOTH ANDREA 1943 Flavicin an antibacterial substance produced by an *Aspergillus flavus* Jour Pharm and Exp Ther 78(2) 164-169
- CHAIN E FLOREY H W JENNINGS M A AND WILLIAMS T I 1943 Helvolic acid, an antibiotic produced by *Aspergillus fumigatus* mut *helvola* Yull Brit Jour Expt Path 24 108-118
- CROWFOOT D M AND LOW B W 1943 A note on the crystallography of helvolic acid and the methyl ester of helvolic acid Brit Jour Expt Path 24 120

- JONES H RAKE G AND HAMRE D W 1943 Studies on *Aspergillus flavus* I Biological properties of crude and purified aspergillic acid Jour Bact 45 461-469
- McKEE C M AND MACPHILLANY H B 1943 An antibiotic substance produced by submerged cultivation of *Aspergillus flavus* Proc Soc Exp Biol and Med 33 (No 2) 217-243
- PHILPOT FLORA J 1943 A penicillin like substance from *Aspergillus giganteus* Wehm Nature 162 725
- WAKSMAN S A AND BUGIE ELIZABETH 1943 Strain specificity and production of antibiotic substances II *Aspergillus flavus-oryzae* group Proc Natl Acad Sci 29 287
- WAKSMAN S A AND CEIGER W B 1943 The nature of the antibiotic substances produced by *Aspergillus fumigatus* Jour Bact 47 391-397
- WAKSMAN SELMAN A AND HORVING ELIZABETH S 1943 Distribution of antagonistic fungi in nature and their antibiotic action Mycol 35 (No 1) 47-65
- WAKSMAN SELMAN A AND ECHATE ALBERT 1943 Strain specificity and production of antibiotic substances Proc Natl Acad Sci 29 (No 2) 74-79 *Aspergillus clavatus* group
- WHITE E C AND HILL J H 1943 Studies on antibacterial products formed by molds I Aspergillic acid a product of a strain of *Aspergillus flavus* Jour Bact 45 433-441
- COOK A H AND LACEY M S 1944 An antibiotic from *Aspergillus parvius* Nature 163 460 April 15
- HOOPER I R AND ANDERSON H W SKELL P AND CARTER H E 1944 The identity of clavacin with patulin Science 93 16
- McKEE C M RAKE G AND HOCK C L 1944 Studies on *Aspergillus flavus* II The production and properties of a penicillin like substance—flavacin Jour Bact 47 187-197
- MENZEL ARTHUR F O WINTERSTEINER O AND HOOGHEIJDE J C 1944 The isolation of ghotoxin and fumagacin from culture filtrates of *Aspergillus fumigatus* J Biol Chem 162 419-429

CHEMISTRY OF MOLD TISSUE

- NORMAN A G PETERSON W H AND HOLTZ R C 1932 I Soluble Carbohydrate Constituents Biochem Jour 26 1934-45
- NORMAN A G AND PETERSON W H 1932 II The Resistant Cell wall Material Biochem Jour 26 1946-53
- PAVENS L M EICHNER E C AND PETERSON W H 1934 III Composition of Certain Molds with Special Reference to the Lipid Content Zentbl Bakt 2 Abt 89 No 17-20 pp 370-377 figs 2
- STRONG F M AND PETERSON W H 1934 IV The Lipids of *Aspergillus sydowii* Jour Amer Chem Soc 56 No 4 pp 952-955
- GORCICA H J PETERSON W H AND STEINBOCK H 1934 V Fractionation of the Nitrogen in the Mycelium of *Aspergillus fischeri* Biochem Jour 28 No 2 pp 504-511
- PRILL E A WENCK P R AND PETERSON W H 1935 VI Factors Influencing the Amount and Nature of the Fat Produced by *Aspergillus fischeri* Biochem Jour 29 No 1 pp 21-33 fig 1
- AROEKER E H STRONG F M AND PETERSON W H 1935 VII The Lipids of *Penicillium aurantio-brunneum* Jour Amer Chem Soc 57 No 2 354-356

- WENCK P R PETERSON W H AND GREENE H C 1935 VIII Innate Factors influencing Growth and Sterol Production of *Aspergillus fischeri* Zentbl Bakt Parasitenk II Abt 92 324-30
- WENCK P R PETERSON W H AND FRED, H B 1935 IX Cultural factors influencing growth and sterol production of *Aspergillus fischeri* Zentbl Bakt Parasitenk II Abt 92 No 13-19 330-338
- WOOLLEY D W STRONG F M PETERSON W H AND PRILL P A 1935 X The phospholipides of *Aspergillus sydowii* Jour Amer Chem Soc 57 No 1? pp 2589-2591
- WOOLLEY D W AND PETERSON W H 1936 XI Isolation of leucine and isoleucine from *Aspergillus sydowii* Jour Biol Chem 114 No 1 pp 85-90
- WOOLLEY D W AND PETERSON W H 1937 XII Isolation of arginine histidine and lysine from *Aspergillus sydowii* Jour Biol Chem 118 No 2 pp 363-370
- WOOLLEY D W AND PETERSON W H 1937 XIII Isolation of some monoamino-monocarboxy and some monoaminodicarboxy acids from *Aspergillus sydowii* Jour Biol Chem 121 No 2 pp 507-520
- WOOLLEY D W AND PETERSON W H 1937 XIV Isolation of cyclic choline sulfate from *Aspergillus sydowii* Jour Biol Chem 122 No 1 pp 213-218 fig 1
- BOHONOS N WOOLLEY D W AND PETERSON W H 1942 XV (Not numbered) The unautolyzable protein of *Aspergillus sydowii* Archives of Biochem 1 (No 2) 319-324
- BOHONOS N AND PETERSON W H 1943 XVI Isolation of fungus cerebrin from the mycelium of *Aspergillus sydowii* Jour Biol Chem 149 295-300

ENZYME PRODUCTION

Enzymes—Aspergillus Flavus-Oryzae Group

- HOFFMANN 1874 Über die Bereitung von Shoyu Saké und Mirin Mitt Gesell Ost 1(6) 8-11
- KELLNER O, MORI Y AND NAGAOKA M 1888 Researches on the manufacture composition and properties of koji Imp College of Agriculture Tokyo Bull 5 9-33
- KELLNER O NAGAOKA M AND KURASHIMA Y 1889 Researches on the manufacture and composition of miso Imp Coll Agr Tokyo Bull 6 1-24
- TAKAMINE J 1894 Process of making diastatic enzyme U S Patent 525 823 Sept 11 other patents in the same diastatic series No 525 825 525 071 562 103 826 699 975 656 991 560 991 561 1 054 676 and 1 054 324
- TAKAMINE J 1894 Process for making alcoholic liquor U S Patent 525 819 525 821 and 525 827
- KELLNER O 1895 Ueber die Bereitung von Saké Shoyu und Miso Chem Ztg 19 120-121
- PRINSEY GEORGE H C 1896 Einige chinesische Sojabohnenpräparate Chem Ztg 20 67-69
- AITA GEN ITSU 1913 Japanische Sojaindustrie Wochsch Brau 30 549-552 559-561 5 illus
- TAKAMINE JOKICHI 1914 Enzymes of *Aspergillus oryzae* and the application of its amylolytic enzyme to the fermentation industry Chem News 110 215-218 In this paper Takamine summarizes the history of the studies of *A. oryzae* and its application to various fermentation industries

- TAKAMINE J 1920 Process for the making of enzyme extracts U S Patent 157 797 Oct 25
- TAKAMINE J JR AND OSHIMA K 1920 The properties of a specially prepared enzymic extract polyzyme comparing its starch liquefying power with malt diastase Jour Amer Chem Soc 42 1261-5
- OSHIMA KOKICHI 1922 Studies on the protease of the *A. oryzae* group and its rôle in shoyu brewing Amer Food Jour 17(1) 30-31
- CHURCH M H 1923 Soy and related fermentations U S Dept Agr Bull 1157 1-27 2 pla 6 figs
- NIISHIMURA S 1925 Zur Kenntnis der Takadiastase Chemie der Zelle und Gewebe 11 207-216
- OSHIMA K 1928 Disinfectants for preserving the amylase solution of *Aspergillus oryzae* Jour Soc Chem Ind 31 70-3 180-3 B
- OSHIMA K 1928 Protease and amylase of *Aspergillus oryzae* Jour Coll Agr Hokkaido Imp Univ 11 135-744
- HABADA T 1931 The preparation of *Aspergillus oryzae* enzymes Ind Eng Chem 23 1424 7
- ITO M 1937 The nature of koji diastase Jour Fac Agr Hokkaido Imp Univ 30(5) 243-366
- WEI H-S AND CHIN H-S 1934 The diastatic activity of *Aspergillus* Science (China) 11 1193-8 *A. oryzae*
- KIRSH D 1935 Lipase production by *Penicillium oxalicum* and *Aspergillus flavus* Botan Gaz 57 320-33
- STUART L S 1935 The production of lipolytic and deplating enzymes by the *Aspergillus flavus-oryzae* group Jour Bact 29 88-9
- TOKUOKA Y 1938 Koji amylase X Effect of external conditions on α - and β -amylases and maltase Jour Agr Chem Soc Japan 14 429-438
- TOKUOKA Y 1938 Koji amylase XI Effect of koji material on production of amylase and maltase Jour Agr Chem Soc Japan 14 839-842
- BABAKINA H G AND ZAMYSLOV A D 1939 Enzyme preparation from *Aspergillus oryzae* in the leather industry Biokhimiya 4 316-26
- NAGATOMO TAKEO 1939 The optimum hydrogen ion concentration for amylase of *Aspergillus oryzae* Jour Agr Chem Soc Japan 15 753-6
- OTANI YOSIO 1939 Enzymes in young mycelia of *Aspergillus oryzae* Bull Agr Soc Japan 16 59-64
- PROSKURYAKOV N I AND OSISOV F M 1939 Enzymic cleavage by molds Biokhimiya 4 50-59 *A. oryzae*
- TOKUOKA Y 1939 Koji amylase XIII Effect of the variety of *A. oryzae* employed in saké brewing upon the formation of amylase maltase and protease in Koji Jour Agr Chem Soc Japan 15 411-418
- UNDERKOFER L A FULMER E I AND SCHOENE LORIN 1939 Saccharification of starchy grain mashes for the alcoholic fermentation industry Use of mold amylase Ind and Eng Chem 31 731-738 *A. oryzae*
- SCHOENE LORIN FULMER E I AND UNDERKOFER L A 1940 Saccharification of starchy grain mashes for the alcoholic fermentation industry Comparison of several saccharifying agents Ind and Eng Chem 32 544-547 (Moldy bran malt and soybean meal)
- HAO LU CHENG 1942 Fungal amylases as saccharifying agents in the ethanol fermentation of starchy materials Doctoral Thesis Ia St Coll Abstracted in Ia St Coll Jour Sci 17(1) 71-73

- HAO, LU CHENG FULMER H I AND UNDERKOPFLER L A 1943 Fungal amylases as saccharifying agents in the alcoholic fermentation of corn Ind Eng Chem 35 814-818 *A. oryzae* and *A. flavus*
- CHRISTENSEN L M 1943 U S Patent 2 325 368 July 27 Apparatus for facilitating the growth of molds on solid substrates as in the large-scale production of highly diastatic bran mold

Enzymes—Aspergillus Niger Group

- BOURQUELOT E 1896 Recherches sur les propriétés physiologiques du maltose Jour Anat et Physiol (Paris) 22 162-204
- FERNBACH, A 1890 Formation de sucrase chez l'*Aspergillus niger* Ann Inst Pasteur 4 1-24
- BOURQUELOT E 1893 Les ferments solubles de l'*Aspergillus niger* Bul Soc Myc France 9 230
- YOUNG V H 1918 Some factors effecting inulase formation in *Aspergillus niger* Plant World 21 75-87 and 114-133
- FUNKE, G L 1922 Researches on the formation of diastase by *Aspergillus niger* van Tieghem Rec Trav Botan Neerl XIX 219-275 14 figures Abstracted in The Review of Applied Mycology 2 Part 7 330-331 1923
- SCHMIDT DOROTHEA 1925 Über die Pilzstärke (amylöse) bei *Aspergillus niger* van Tieghem und einige Bemerkungen über ihren diastatischen Abbau Biochem Ztschr 258 223-232
- FUNKE G L 1926 Researches on the formation of diastase by *Aspergillus niger* Rec Trav Bot Neerland XXIII 200
- BERNHAEUER K 1928 Beiträge zur enzymchemie der Durch *Aspergillus niger* bewirkten Säurebildungsvorgänge I Ztschr Physiol Chem 177 86-106
- MÜLLER D 1931 Glucos oxidase. IV. Glucose oxidase from *A. niger* Biochem Zeit 232 423
- ROSSI G AND SCANDELLARI G 1932 Influence of salts on invertase formation in *Aspergillus niger* Atti IV Congr Naz Chim Pura Applicata 1933 795-9
- MIWA T AND YOSHII E 1934 The formation of urease by *Aspergillus niger* Science Repts Tokyo Bunrika Daigaku I 243-70
- OTANI H 1934 Sulfopeptidase of molds I *Aspergillus niger* Acta Schol Med Univ Imp Kyoto 17 242
- DRBOGLAY M A AND KIRSANOVA B A 1935 A study of the invertase of *Aspergillus niger* Proc Inst Sci Res Food Ind (Leningrad) 3 115-129
- MESSADROLI G AND AMATI A 1935 Action of various alkaloids on the sucrase produced by *Aspergillus niger* Atti Accad Lincei 21 46-50
- OTANI H 1935 The proteolytic enzymes of *A. niger* Acta Schol Med Univ Imp Kyoto 17 317-322
- ROSSI G AND SCANDELLARI G 1935 The influence of different salts contained in Wehmer's liquid medium on the quantity of invertase produced by *Aspergillus niger* growing in this medium Biochim Terap Sper III 87-91
- DRBOGLAY M A AND KIRSANOVA B A 1936 Peroxidase activity of *Aspergillus niger* Proc Inst Sci Res 3 103-110
- DRBOGLAY M A AND KIRSANOVA B A 1936 Amylase activity of *Aspergillus niger* Proc Inst Sci Res Food Ind (Leningrad) 3 4 112-15
- PROVIN E I AND BLINNKOVA F I 1936 The thermostability and regeneration of amylase from *Aspergillus niger* Arch Sci Biol USSR III 3 11-14

- MOOR J C 1939 The enzymes of *Aspergillus niger* which transform alanine and aspartic acid Proc Acad Sci Amsterdam 42 195-200
- MÜLLER D 1940 Glucose oxidase Naturwissenschaften 27 516 *A. niger*

Enzymes—Aspergillus General

- DOX A W 1919 The intracellular enzymes of *Penicillium* and *Aspergillus* U S Dept Agr Bur Anim Ind Bul 120 70 pp
- HOPPE ANNA 1919 Ueber einen bisher unbekannten celluloselösenden im Verdauungstraktus vorkommenden *Aspergillus A. cellulosa* seine Zucht und seine Eigenschaften Centralb f Bakt etc 1 Abt 83 531-537
- COMPTON A 1921 Studies in the mechanism of enzyme action I Role of the reaction of the medium in fixing the optimum temperature of a ferment Roy Soc London Proc Ser B 92 no B642 pp 1-6 figs 3
- WAKSMAN S A 1922 Enzymes of Microorganisms Abstr Bact 6 265-299 331-360
- WAKSMAN S A 1922 Use of enzymes in the clarification of jellies and fruit juices Canner 54(18) 4-46
- OSHIMA K AND CHURCH MARGARET B 1923 Industrial mold enzymes Jour Ind Eng Chem 15 6
- OWEN W L 1923 Summarizes his many years study on this subject in Mold fungi in sugar inversion Facts about Sugar 18 519-521 546-548
- MÜLLER D 1928 Studien über ein neues Enzym Glykoseoxydase frühere Untersuchungen über die Wirkung von Enzympräparaten aus *Aspergillen* auf Monosaccharide und organische Säuren Biochem Zeitschr 199 136-70
- IVANOV N N AND AVETISOVA A N 1931 The enzymic transformation of guanidine to urea Biochem Zeit 231 67-78
- ETRE J C 1932 Cultural studies on *Aspergillus* with special reference to lipase production of strains isolated from stored copra and cacao Ann Appl Biol 19 351-69
- HOFMANN L 1934 Enzymes from molds which hydrolyse glucosides and diaccharides Biochem Zeit 273 199-206
- JOHNSON M J AND IYERSON W H 1935 The proteinase and aminopolypeptidase of *Aspergillus parasiticus* Jour Bact 25 90 Abstract
- KRISH D 1935 Factors influencing the activity of fungus lipase J Biol Chem 108 421-30
- MIHAELOFF S 1935 Soluble enzymes secreted by *Aspergillus fumigatus* Bull Inst Egypte 17 163-66
- OTANI H 1935 The Mold Enzymes Splitting Nucleic Acid Acta Schol Med Univ Imp Kyoto 17 323-329 *A. niger* and *A. oryzae*
- GRASSMANN W AND RUBENBAKER H 1937 Enzymes from molds U S Patent 2 102 315 December 14 C A 32 1991
- MIWA TOMO AND MIWA ATAKO 1940 α glucosidase II Enzymes of mold fungi Jour Chem Soc Japan 61 1179
- PEARCE A A 1940 On the so-called Iodide Oxidase Mechanism of Iodide Oxidation by *Aspergillus* Biochem Jour 34 1493-1500 *A. niger*
- UNDERKOFER L A 1942 Microbial amylases—Their application to alcoholic fermentation Brewers Digest 17 29 A review with 25 references
- AYERS G B AND TORIE W C 1943 The isolation of crude proteolytic enzymes

- GUÉGUEN FERNAND 1909 *Aspergillus fontynonis* nova sp parasite probable des nodosités juxta articulaires Comp Ren Soc Biol III 1052 1053 also 67 10-12
- GUÉGUEN FERNAND 1911 *Abrès sous dermiques à répétitions produits par l'Aspergillus fontynonis* n sp morphologique et biologique de cette espèce Arch Parasitol 14 177-192
- QUEVEDO JOSÉ M 1912 Sur une encephalo myelite épizootique d'origine mycosique chez les chevaux Premier Congr Internat Path Comp 2 200-202
- QUEVEDO J M 1912 Estudio de un *Aspergillus* patogeno Di Agronomia 3 1-36
- STOUT A B 1912 A fungous infection of the ear Jour N Y Bot Garden 13 196-7
- DE SCHWEINITZ GEORGE E 1915 Kerato mycosis Proc Path Soc Phila n s II 19
- HOLLANDE A C R AND BEAUVERIE J 1916 Spirales de Curchmann et *Aspergillus pulmonaire* Bul Soc Myc France 32 17-24
- CITABLE ROBERT 1917 Über die pathogene Wirkung des *Aspergillus fumigatus* in der Subkutis der Meerschweinchen Arch Derm u Syphilis 124 14
- CASTELLANI A AND CHALMERS A J 1919 Manual of Tropical Medicine ed 3 London pp 1026-1032
- EMILE WEIL P AND GAUDIN L 1919 Contribution à l'étude des onychomycoses IV Onychomycoses causées par *Sterigmatocystis unguis* Archives Méd Exp Anat Path Paris 28 463-465 illust
- HARVER DOUGLAS AND JOCKEY T 1919 Specimens of *Aspergillus fumigatus* from nasal sinuses Proc Roy Soc Med (London) 12 Laryngology 187-188
- SARTORY ANTOINE AND FLAMENT L 1920 Étude morphologique et biologique d'un *Aspergillus* nouveau isolé d'expectorations d'un malade suspect de tuberculose pulmonaire Compt Rend Soc Biol 93 1114-1115
- GALAVILLE AND CAERLST 1922 Un cas d'otomycose aspergillaire Bull Sci Pharmacol XXIX nos 8-9 p 438-439
- LANGERON MAURICE 1922 Sur un champignon d'une otomycose brésilienne *Sterigmatocystis Hortai* Bull Soc Path Exot 16 383 384
- CHURCH MARGARET B AND BLACKLEY JOHN S 1923 Laboratory feeding of molds to animals Amer Vet 4 7-13
- GARDEY F 1923 Aspergillosis of the lung Semana Medica Buenos Aires 1 300
- LYNCH K M 1923 *Aspergillus* in scalp lesions following red bug (*Leptus*) bites Archives Derm and Syph 7 599
- OTA MASAO 1923 Sur une nouvelle espèce d'*Aspergillus* pathogène *Aspergillus Jeansemetii* n sp Ann Parasitol Hum Comp 1 137-146
- SARTORY A 1920 to 1923 Champignons Parasites de l'Homme et des Animaux In fascicles
- SARTORY A AND BAILLY A 1923 Les mycoses pulmonaires et leurs parasites Paris p 335
- VINCENS F 1923 Sur l'aspergillomycose des abeilles Compt Rend Acad Sci (Paris) 177 540-542
- CLELAND J H 1924 Aspergillosis of pleura with sclerodium formation Med Jour Australia (Sydney) 1 634
- LANGERON MAURICE 1924 Un *Sterigmatocystis* nouveau parasite de l'homme en Tunisie *S. tunetana* n sp Bull Soc Path Exot 17 315-317 1 fig

- MACFARLAN D 1924 Fungus on Tongue Jour Amer Med Assn 83 1-35
- ANDERSON C 1923 Sur un cas d otomycose à *Sterigmatocystis nigra* Arch Inst Pasteur Tunis 14 93-96
- VAN LEEUWEN W STORM BIEV Z KREMER W AND VAREKAMP H 1923 Leber die Bedeutung kleinporigen Aspergillus-Arten (Typus *Aspergillus fumigatus*) für die Aetiologie des Asthma bronchiale Ztschr Immunitätsforsch Bd 44 1-25
- LAPHAM MARY E 1926 Aspergilliosis of the lungs and its association with tuberculosis Jour Am Med Assoc 87 1031-1037
- SARTORY ANTOINE SARTORY R AND MEYER JACQUES 1926 La formation des périthèces chez *Aspergillus fumigatus* Fresenius sous l'influence du radium Compt Rend Acad Sci 183 1360-1367
- STEELE ALBERT F 1926 A case of infection with *Aspergillus terreicolor* Boston Med Surg Jour 195 536-538
- PINOT E AND NANTA A 1927 Aspergilliose expérimentale chez le lapin Compt Rend Soc Biol 97 67-8
- PINOT F AND NANTA A 1927 Sur l'existence fréquente d'une mycose de la rate en Algérie Compt Rend Acad Sci 184 31-8
- VAN LEEUWEN W STORM FINTHOVEN W AND KREMER W 1927 The allergen proof chamber in the treatment of bronchial asthma and other respiratory diseases The Lancet 212 1237-9
- VAN LEEUWEN W STORM AND KREMER W 1927 Schimmelpilzallergene als Krankheitsursachen Klin Wochenschrift 6(9) 1-9
- MAJSET A BERTRAND J AND MAGNOR J 1928 Recherches expérimentales sur l'aspergilliose splénique Compt Rend Soc Biol 98 760-70
- HANSEN A 1928 Ueber Schimmelpilz Asthma Verhandl d Deutsch Gesellsch f Inn Med Hong 40 204-6
- KELLER PH 1928 Epidermal Aspergillusmykose der Haut Dermat Wochen schr 87 1831-3
- MATTA ALFREDO DA 1928 *Sterigmatocystis tropicalis* n. sp. de fungo patogenico para o homem Bol Inst Brasil Sci 3 51-54 1 pl
- NANTA A AND CHATELLIER L 1928 Aspergilliose cutanée et splenomycose Bull Soc Franç de Dermat et Syph 35 671-3
- WAHL E F AND ERICKSON M J 1928 Primary pulmonary aspergilliosis Jour Med Assn Georgia 17 241-9
- COCCHERI P 1929 Micon pulmonare da *Sterigmatocystis nigra* van Tieghem Atti Ist Bot R Univ Pavia IV 1 161-181 8 figs
- PUESTOW K L 1929 Maduromycosis a contribution to the study of maduromycosis with report of a case of infection with *Aspergillus nidulans* Arch Derm Syphilol 20 642-664
- AGOSTINI ANGELA 1930 Dermatormicon dovuta a *Eurotium rubrum* Bremer Atti Ist Bot R Univ Pavia IV 2 65-9 5 figs
- BERNSTON HARRY S 1930 Asthma due to a mold—*Aspergillus fumigatus* Jour Am Med Assn 95 189-190
- DA FONSECA OLYMPIO 1930 Mycetoma por *Aspergillus Amsterdamii* Jour Med Cir Brasil 38 415-430 Pl I (1-19)
- TALICE R V AND MCKINNON J E 1931 *Aspergillus (Eurotium) montevideensis* n. sp. isolé d'un cas d'otomycose chez l'homme Soc de Biol (Paris) Compt Rend 108 1007-1009
- BERNSTON HARRY S AND THOM CHARLES 1933 The importance of molds as allergic excitants in some cases of vasomotor rhinitis Jour Allergy 4 114-122

- STEINBERG R A 1936 Relation of accessory growth substances to heavy metals, including molybdenum in the nutrition of *Aspergillus niger* Jour Agr Res 55 439-48
- STEINBERG R A 1936 Effects of barium salts on *Aspergillus niger* and their bearing on the sulfur and zinc metabolism of the fungus in an optimum solution Botan Gaz 57 666-71
- BUTKEVICH V S AND TROFIMOVA E I 1937 Magnesium as an activator of bio chemical conversions Compt Rend Acad Sci (USSR) (In English) 17 221-5
- STEINBERG R A 1937 Role of molybdenum in the utilization of ammonium and nitrate nitrogen by *Aspergillus niger* Jour Agr Res 55 891-907
- BATES JAMES C 1938 Effects of certain alkaloids on the growth of *Aspergillus niger* and *Rhizopus nigricans* Univ Kansas Sci Bull 28 85-112
- STEINBERG R A 1938 The Essentiality of Gallium to Growth and Reproduction of *Aspergillus niger* Jour Agr Res 57 569-574
- STEINBERG R A 1938 Correlation between Biological Essentiality and Atomic Structure of the Chemical Elements Jour Agr Res 57 851-858
- TAUSOV V O 1938 The conversion of energy by microorganisms VIII The amount of living and of dead cells in mold fungi Microbiology (USSR) 7 75
- STEINBERG R A AND BOWLING J D 1939 Optimum solutions as physiological reference standards in estimating nitrogen utilization by *Aspergillus niger* Jour Agr Res 58 717-732
- STEINBERG R A 1939 Effects of nitrogen compounds and trace elements on growth of *Aspergillus niger* Jour Agr Res 58 731-743
- STEINBERG R A 1939 Relation of carbon nutrition to trace-element and accessory requirements of *Aspergillus niger* Jour Agr Res 59 749-763
- KAUFFMANN COSLA O VASILIU N AND BRULL R 1940 Effect of ions on the germination and development of the spores of *Aspergillus niger* Rev Gen Botan 22 97
- STEINBERG R A 1940 Action of some organic compounds on yield sporulation and starch formation of *Aspergillus niger* Jour Agr Res 60 765-773
- TAMITA H AND USAMI S 1940 Growth of *Aspergillus oryzae* with the addition of amino acids as the only source of carbon and nitrogen Acta Phytochim (Japan) 11 261-98 (in German)
- GARREAU Y 1941 Formation of sulfuric acid from some organic sulfur derivatives by *Aspergillus niger* Compt Rend Soc Biol 135 508
- IVANOV N N AND MAKRIKOVA N A 1941 The influence of acenaphthene on formation of organic acids in *Aspergillus niger* Doklady Akad Nauk USSR 30 356-8
- STEINBERG R A 1941 Sulfur and trace-element nutrition of *Aspergillus niger* Jour Agr Res 59 103-27

PIGMENTS AND COLORING SUBSTANCES

- LINossier GEORGES 1891 Sur une hématine végétale l'aspergilline Compt Rend Acad Sci (Paris) 112 807-808
- KLÖCKER A 1916 Über die Bildung eines Fluorescein ähnlichen Stoffes in Kulturen von *A. glaucus* Zentbl f Bakt 2 Abt 46 225-6
- BLOCHWITZ A 1928 Farbenänderung Verschiedenfarbigkeit Farbenvariation bei Schimmelpilzen Ber Deuts Bot Ges 48 516

- Metz O 1930 Über Wachstum und Farbstoffbildung einiger Pilze unter dem Einfluss von Eisen, Zink und Kupfer *Arch Mikrobiol* 1 19 21
- BLOCHWITZ A 1931 Die Farbstoffe der Schimmelpilze *Zentralbl Bakt* II 80 201
- RAI TRICK H 1937 Biochemistry of the lower fungi *Fungal disease der Enzymforschung* 1 345-363
- QUILICO A AND DI CAPUA A 1933 Aspergilline the pigment of *Aspergillus niger* spores I *Vita Acad Lincei* 17 97-8
- QUILICO A AND DI CAPUA A 1933 Aspergilline the pigment of *Aspergillus niger* spores II *Vita Acad Lincei* 17 177-82
- GOLD B S AND RAI TRICK H 1931 The biochemistry of microorganisms *NL chem Jour* 28 1610-17
- RAI TRICK H ROBINSON ROBERT AND TOLD I R 1933 The chemistry of Aspergillus colouring matters Part I (London) *Chem Soc Jour* 1937 50-58
- RAI TRICK H ROBINSON ROBERT 1932 The chemistry of Aspergillus colouring matters Part II (London) *Chem Soc Jour* 1938 206-2064
- CHICK HICK J H RAI TRICK H AND ROBINSON ROBERT 1932 The chemistry of Aspergillus colouring matters Part II (London) *Chem Soc Jour* 1938 206-2064
- LAVALLEY J AND LABORET F 1933 The circumstances of the appearance of yellow pigments in the liquid culture of *Aspergillus niger* *Compt Rend* 208 1045-6
- LAVALLEY J RAI TRICK H AND RICHARDS T 1939 The biochemistry of microorganisms *LAM The crystalline coloring matters of species in the Aspergillus glaucus series* 2 *Biochem Jour* 33 1301 1303
- VOLLAT J AND LABORET F 1939 Characterization of lactoflavin produced by *Aspergillus niger* v Tgh partially deficient in magnesium *Compt Rend* 208 1046-8
- KNOBLOCH H AND SELLMANN R 1941 The formation of flavin type pigments in liquid cultures of *Aspergillus niger* *Zentralbl Bakt Parasitenk* 103 77
- SOIL TESTS FOR MINERAL DEFICIENCIES
- SCHLOTS F F SMITH I B AND BROWN P F 1932 *Aspergillus niger* as an indicator of available phosphorus in the soil *Iro Iowa Acad Sci* (1931) 38 303-307
- MEHLICH A TALOG E AND FRYD E B 1933 The *Aspergillus niger* method of measuring available potassium in soil *Soil Science* 35 2-9
- NIKLAS H POSCHENBERGER H AND TRISCHLER J 1933 Urteile und Erfahrungen über die Verwendbarkeit und Brauchbarkeit der *Aspergillus*-Halbmethoden und deren Beurteilung nach dem Stand der bisherigen Forschungsergebnisse *Ztschr Pflanzenern Düng und Bodenk* 12 3 102-130
- STOCK JUREK 1933 Kulturversuche mit *Aspergillus niger* als Indikator für die Hungerbedürftigkeit *Bot Arch* 35 1-6
- BUTKEVICH I S AND NADIRYAN O G 1934 Microbiological methods in determining the fertilizer requirements of soils *Chemisation Societate Agr No 4* 62-60
- NIKLAS H 1934 Bodenuntersuchungsmethoden Die *Aspergillus* methode von Niklas und Mitarbeitern *Ztschr Pflanzenern Düng und Bodenk* 13 64-65
- SMITH A M AND DRYBURG A 1931 The examination of soils by means of *Aspergillus niger* *Jour Soc Chem Ind* 53 50-254

VITAMINS AND GROWTH SUBSTANCES

- BOYSEN JENSEN P 1931 Formation of a growth regulator by *Aspergillus niger*
 Biochem Zeit 239 213-9
- SCHOPMEYER H AND FULLER F I 1931 The production of yeast growth stimulants by the molds I *Aspergillus niger* *Trichoderma lignorum* and *Aspergillus clavatus* Jour Bart 22 23-28
- SAKAMURA T AND YANAGIHARA T 1932 The production of the growth substance by *Aspergillus niger* Proc Imp Acad Tokyo 8 397-9
- SCHOPMEYER H 1932 Production of yeast growth stimulants by molds on various media Ia St Coll Jour Sci 6 471-2
- BUNNING E 1934 Growth and nitrogen assimilation in *Aspergillus niger* under the influence of growth regulators Ber Deut Botan Ges 51 423-44
- BERNHARTER A AND CORLICH B 1936 The formation of vitamin C like substances by fungi and bacteria I Biochem Zeit 286 80
- KOMAROV S N 1936 Methods of obtaining an antirachitic preparation from the waste products of citric acid manufacture from the mycelium of *Aspergillus niger* Proc Sci Inst Vitamin Res USSR 1(2) 162-71
- OPPEL V V 1936 Chemistry and biochemistry of flavins Vitamins and vitaminization Proc Sci Inst Vitamin Res USSR 1(2) 5-57
- SCHFLUMERT A AND SCHIEBLICH M 1936 Vitamin production by *Aspergillus oryzae* Biochem Zeit 286 66-71
- FUKUMOTO J AND SHINOMURA H 1937 Formation of Vitamin C like substance by molds Jour Agr Chem Soc Japan 13 613-20
- KITAVIN G S 1939 Action of mercury salts on the formation of vitamin B₂ in *Aspergillus niger* Biokhimiya 4 283-94
- LAYOLLA J AND LABOREY F 1939 Characterization of lactoflavin produced by *Aspergillus niger* v Tgh partially deficient in magnesium Compt Rend Acad Sci 208 1036-8
- SAKURAI KYUYA 1939 Vitamin B₂ synthesis by microorganisms I Molds Jour Sci Hiroshima Univ Ser B 2 3 191-200
- KITAVIN G S 1940 Crystalline riboflavin obtained through the action of mercuric salts on *Aspergillus niger* Compt Rend Acad Sci USSR 28 51, 18
- SAKURAI K 1940 Vitamin synthesis by microorganisms II Molds Jour Sci Hiroshima Univ Ser B 2 4 1-6
- KNOBLOCK H AND SELLMANN R 1941 The formation of flavin type pigments in liquid cultures of *Aspergillus niger* Zent Bakt Parasitenk II Abt 103 777-80
- EAKIN R I AND EAKIN E A 1942 Biosynthesis of Biotin Science 96 187
A. niger synthesizes biotin from biotin free media

MISCELLANEOUS PRODUCTS

Alcohol

- MOLLARD M 1916 Catalytic rôle of potassium nitrate in alcoholic fermentation produced by *S. nigra* Compt Rend Acad Sci (Paris) 163 50-52
- YCELL J L 1928 Alcoholic fermentation by *Aspergillus flavus* Brefeld Biochem Jour 22 1501-7
- SAKAGUCHI K AND YAKANO M 1932 Alcohol fermentation by *Aspergillus oryzae* Jour Agr Chem Soc Japan 6 115-22
- WATKIN V 1940 New process for making alcohol from chicory Spirto Vodoch naya Prom 17(9) 13-14

Chitin

- NORMAN A G AND PETERSON W H 193 The chemistry of mold tissue II
The resistant cell wall material Biochem Jour 28 1916-53

Ergosterol

- PREUSS L M PETERSON W H AND FRED I B 1937 Isolation and identification of ergosterol and mannitol from *Aspergillus fischeri* Jour Biol Chem 127 483-9

Fluorescein

- KLOECKER A 1917 Formation of a substance resembling fluorescein in the cultures of *Aspergillus glaucus* Compt Rend Trav Lab Carlsberg M 312-313

Gum

- SANBORN J R 1934 Microbiological film production Ind Eng Chem 26 532-3
SANBORN J R 1935 Sheet material and method of manufacturing the same U S Patent 2 076 253
SANBORN J R 1936 Gums produced by fungi industrial utilization Ind Eng Chem 28 1189-1190

Hydroxylamine

- LEMOIGNE M AND DESVAULT R 1935 Formation of hydroxylamine in cultures of *Sterigmatocystis nigra* in a medium rich in ammonium nitrate Compt Rend 201 239-41
LEMOIGNE M AND DESVAULT R 1936 Recherches sur la rôle biochimique de l'hydroxylamine I Formation de l'hydroxylamine par le *Sterigmatocystis nigra* sur des milieux au nitrate d'ammonium Bull Soc Chem Biol 16 604-614

Mannitol

- BRACONNOT H 1813 Nouvelles recherches analytiques sur les champignons pour servir de suite à celles qui ont été insérées dans les tomes LXXX et LXXX des Annales de chimie Ann Chim 87 231-240 (Cited by Birkinshaw et al 1931)
VAUCQUELIN 1813 Expériences sur les champignons Ann Chim 85 1-25 (Cited by Birkinshaw et al 1931)
BIRKINSHAW J H CHARLES J H V HETHERINGTON A C AND RAINTRICK H 1931 Studies in the biochemistry of micro organisms IX On the production of mannitol from glucose by species of *Aspergillus* Trans Roy Soc London 220B 153-171
PREUSS L M PETERSON W H AND FRED I B 1937 Isolation and identification of ergosterol and mannitol from *Aspergillus fischeri* Jour Biol Chem 127 483-9
YAMASAKI I AND SHIMOMURA M 1937 Formation of d mannitol from glycerol by molds of the *Aspergillus glaucus* group Biochem A 291 310-8

Polysaccharides

- KOSTYCHEV S 1930 Formation of sugar by molds III *Physiol Chem* 111 236
- NORMAN A G PETERSON W H AND HOLTZ R C 1932 The chemistry of mould
 ti suc I Soluble carbohydrate constituents *Biochem Jour* 26 1934-45
- HIDA T 1934 Über die Starkebildung von Schimmelpilzen *Jour Shanghai
 Sci Inst* 1 85-116
- CLUTTERBUCK P W 1936 Recent developments in the biochemistry of the fungi
Jour Soc Chem Ind 55 55T 61T

Ochracin

- YABUTA T AND SUMIYAMA Y 1934 Chemical constitution of ochracin (a fermenta-
 tion product of *Aspergillus ochraceus*) II *Jour Agr Chem Soc Japan* 10
 703-14

Terrein

- RAISTRICK H AND SMITH G 1935 Biochemistry of micro-organisms XLII
 The metabolic products of *Aspergillus terreus* Thom A new mold metabolic
 product—*Terrein* *Biochem Jour* 29 606-11

CHAPTER XXIII

GENERAL BIBLIOGRAPHY

- ABBOTT E V 1906 Taxonomic studies on soil fungi Iowa State Coll Jour S 1 15-36
- ALBERG C L AND BLACK O F 1913 Contributions to the study of mouse deterioration U S Dept Agr Bur Plant Ind Bul 270 1-49
- AMANN J 1906 Conservierungsfähigkeiten und Einschlußmedien für Moore Chloro und Cyanophyceen Zeitsch f Mikr opie III 19-21
- AMO W J TH 1901 Bijdrage tot de kennis van de flora van het teruggaan le suiker Archief voor de Suikerindustrie in Nederland I In die III 4-19
- ANSLOW WINSTON K AND RAISTRICK HAROLD 1935a The biocentrism of microorganisms LVII Fumigatin and spinulosin metabolic products respectively of *Aspergillus fumigatus* Fres and *Penicillium spinulosum* Thom Biochem Jour 22 68-96
- ANSLOW WINSTON K AND RAISTRICK HAROLD 1935b The biocentrism of microorganisms LVIII Spinulosin a metabolic product of a strain of *Aspergillus fumigatus* Fresenius Biochem Jour 22 228-99
- ASHLEY JULIUS NICHOLSON AND RAISTRICK HAROLD AND RICHARDS FALLESIN 1939 The crystalline colouring matters of species in the *Aspergillus glaucus* series Part II Biochem Jour 33 1291-1303
- BAINTER G 1931 Sur quelques espèces de Sterigmatocystis Soc Bot de France pp 6-9 February
- 1908a Mycothèque de l'école de pharmacie XXIV XXXII Bul Soc Mycol France 24 73-91 Plate VIII figs 1-13
- AND SARTORY A 1908b Étude d'un *Aspergillus* pathogène *Aspergillus fumigatoides* Compt Rend Soc Biol 66 27-33
- AND SARTORY A 1909 Étude d'un *Aspergillus* pathogène (*Aspergillus fumigatoides* n sp) Bul Soc Mycol France 25 111-118 illus Pl V
- AND SARTORY A 1911a Étude d'une espèce nouvelle de Sterigmatocystis *Ster flaripes* nov sp Bul Soc Mycol France 27 90-96 Plate III figs 1-6
- AND SARTORY A 1911b Études biologiques et morphologiques de certains *Aspergillus* Bul Soc Mycol France 27 95-107 316-318
- AND SARTORY A 1911c Étude biologique et morphologique de certains *Aspergillus* à pigment (suite) Bul Soc Mycol France 27 453-463 illus
- AND SARTORY A 1912a Étude de quelques Citromyces nouveaux Bull Soc Mycol France 28 38-49
- AND SARTORY A 1912b Étude biologique et morphologique de certains *Aspergillus* Bul Soc Mycol France 28 23-269 illus 1 schéma A umbrosus
- AND SARTORY A 1913 Étude d'une espèce nouvelle de Sterigmatocystis *Sterigmatocystis sydowii* n sp Ann Mycol 11 23-29 Plate III
- BARNES B 1928 Variations in *Furotum herbariorum* (Wigg) Link induced by the action of high temperatures Ann Bot (London) 42 563-512 illus
- BARY A DE 1854 Ueber die entwicklung und den zusammenhang von *Aspergillus glaucus* und *Furotum* Bot Ztg III 423-434 441-451 463-471 illus

- 1870 Furotium Erysiphe Cinnaboli Nebst Bemerkungen über die geschlechtsorgane der ascomyceten I. Furotium Senckenb Naturf Gesell Abhandl 7 361-382 illus
- BERKELEY M J 1857 Introduction to cryptogamic botany 601 pp illust Bailliere London
- BERNHAEUER A 1929 Ueber die Charakterisierung der Stämme von *Aspergillus niger* auf Grund ihres biochemischen Verhaltens I Vergleichende Untersuchungen über die Säurebildung durch verschiedene Pilzstämme Biochem Zeitschr 197 2, 8-86
- AND PATZLT GEORGE 1935 Über Schimmelpilzsterine I Mitteilung Die Sterinbildung bei *Aspergillus niger* Biochem Zeit 280 388-93
- AND POSSELT GRFTE 1937 Über Schimmelpilzlipide II Mitteilung Die Zusammensetzung eines *Aspergillus niger* Fettes Biochem Zeit 294 215-20
- BERNTOX HARRY E 1930 Asthma due to mold—*Aspergillus fumigatus* Jour Am Med Assn III 189-190
- BEZSSONOFF N 1919 Über das Wachstum der Aspergillaceen und anderer Pilze auf stark zuckerhaltigen Nahrboden Ber deut Bot Gesellsch 36 646-648
- BLAKESLEE A F 1915 Lindner's roll tube method of separating cultures Phytopathology 5 68-69 Pl VII
- BLOUZE PH 1923 Les moisissures du groupe *Penicillium* Link Étude Monograph La Cellule III 1-330 Col Pl 13 figs 1-137
- 1939 Brevis conspectus generis *Aspergillus* Link Manuscript prepared for presentation at the Third International Microbiological Congress in New York Unpublished Copy at the Northern Regional Research Laboratory Peoria Illinois
- BLOCHWITZ ADALBERT 1925 Entstehung von *Aspergillus* varietäten mit verschiedenen konidientragern Ber deut Bot Ges 45 105-108
- 1928 Farbenänderung Verschiedenfarbigkeit und Farbenvariation bei Schimmelpilzen Ber deut Bot Ges 46 516-24
- 1929a Die gattung *Aspergillus* Neue Spezies Diagnosen Synonyme Ann Mycol 27 205-240 illus
- 1929b Die Aspergillaceen Ann Mycol 27 185-240 illust
- 1930 Standorte und geographische Verbreitung der Schimmelpilze Ann Mycol 28(3/4) 241-268
- 1931 Luftmyzelbildungen bei Schimmelpilzen Bot Centralb Beihefte Abt Anal und Phy 48 176-182
- 1932a Perithezien Sklerotien und Endosporische Blasen der Aspergillaceen Beih z Bot Centr Abt I 49 262-292
- 1932b Die Urformen der Aspergillen Hedwega 72 173-174
- 1932c Variabilität und Vererbung bei Schimmelpilzen Deut Bot Gesell Ber 50 218-256
- 1933 Die Gattung *Aspergillus* Neue Spezies Synonyme und Nachträge Ann Myc 31 13-83
- 1934 Die Gattung *Aspergillus* III Neue Spezies Varianten und Mutanten der Konidienfarbe Synonyme und interessante Standorte Ann Mycol 32 83-89
- 1935 Die Urformen der Aspergillen II Bot Centralb Beihefte Abt Morph and Physiol 53 45-50
- BOEDHJ H B 1928 Notes on some *Aspergilli* from Sumatra Ann Mycol 26 69-84

- BOUV P R 1931 Mechanism of the synthesis of fats at the expense of sugars
Compt Rend 193 411-2
- BORZI A 1884 *Inzengera* ein neuer Ascomycet Prugab im Jahrb Wiss Bot
18 450-463 pl 19 '70
- BREFELD ■ 1874 Botanische untersuchungen über Schimmelpilze Heft ■
Leipzig
- 1876 Neue kulturmethoden für die untersuchung der pilze : Sitzungsber
d Ges naturf Freunde zu Berlin 12-33
- 1876 Mycologische untersuchungen Sitzungsber der Versamml deut
Naturforscher und Aerzte zu Hamburg im September Bot Ztg 35 (1877)
77-80
- BULLER A H REGINALD 1933 Researches on fungi V pt 1 pp 1-167
- BUSH M T AND GOTH A 1913a An antibacterial substance produced by an
Aspergillus flavus Federation Proc 2 75
- BUSH M T AND GOTH A 1913b Flavicin an antibacterial substance produced
by an *Aspergillus flavus* Jour Pharm and Exp Ther 75(2) 164-169
- CALAM C T OXFORD A F AND RAISTRICK H 1939 The biochemistry of micro
organisms LXIII Itaconic acid a metabolic product of a strain of *Aspergillus terreus* Thom Biochem Jour 33 1485-1490
- CENI C 1905 Potere patogeno dell *Aspergillus ochraceus* e suo rapporto coll etio
logia e patogenesi della pellagra Riv sper d freniat Reggio Emilia 31
also Arch Ital Mal Nerv Ment 42 505-617 P II
- Centraalbureau voor Schimmelcultures 1939 List of cultures 137 pp Baarn
Netherlands
- CHAUDHURI H AND SACHAR G S 1934 A study of the fungus flora of Punjab
soils Ann Mycol 11 90-100
- CHOWDHURY H P AND MATHUR R S 1938 On a new species of *Emericella* found
in Lucknow Ann Myc 36 61-63
- CIFERRI R 1938 Ritrovamento e cultura dell *Emericella varicolor* Berkeley
(Eurotiaceae) Nuovo Giorn Bot Ital 45 159-173 pl 6
- COOK A H AND LACEY M S 1944 An antibiotic from *Aspergillus parasiticus*
Nature 163 (April 15) 460
- CORDA A C I 184 A glaucus var repens Icones Fungorum 6 ■ illus
- COSTANTIN J AND LUCET A 1903 Sur le *Sterigmatocystis pseudo nigra* Bull
Soc Myc France 11 33
- COSTANTIN J AND LUCET A 1903 Recherches sur quelques *Aspergillus* patho
g'nes Ann Sci Nat Bot IV 2 119-180 pl 5
- CRAMER C 1859 Vierteljahresschrift der Naturforsch Gesellschaft Zurich 4
325
- CRUICKSHANK J H RAISTRICK H AND ROBINSON ROBERT 1933 The chemistry
of *Aspergillus* colouring matters Part II (London) Chem Soc Jour
1938 206-2064
- CORZI M 1934 Un aspergillacea ad ascospore stellate Rend Accad Naz
Lincei 19 421-428 fig 1
- CZAPEK F 1907 Untersuchungen über die stickstoff gewinnung und Eiweiss
bildung der Pflanzen Beiträge zur Chemischen Physiologie und Pathologie
Band 1 Heft 10-12 pp 540-560 also Band 3 pp 47-66 1903
- DALE E 1907 On the morphology and cytology of *Aspergillus repens* DeBary
Ann Mycol 7 215-225
- 1912 On the fungi of the soil Ann Mycol 10 432-447
- 1914 On the fungi of the soil II Ann Mycol 11 33-67

- DANGEARD P A 1907 L'origine du périthèce chez les ascomycètes Le Botaniste 10 1-385
- DELACROIX G 1893a Espèces nouvelles observées au Laboratoire de Pathologie végétale Bul Soc Mycol France 9 184-188
- 1903b Champignons parasites nouveaux Bul Soc Mycol France 9 264-268
- DEMAZIERES J 1934 Descriptions et figures de six Hyphomycetes inédites à ajouter à la Flore française Ann d Sci Nat 2 Ser Bot 2 69-73
- DODGE C W 1935 Medical Mycology 900 pp illus Mosby and Company St Louis
- DOX A W 1910 The intracellular enzymes of *Penicillium* and *Aspergillus* with special reference to those of *P. camemberti* Bul U S Dept Agr Bur Animal Ind 120
- FIDAM F 1883 Zur Kenntniss der Entwicklung bei den Ascomyceten III *Sterigmatocystis nidulans* n. sp. Cohn Beitr Biol Pflanzen 3 39-411 pl 20-72
- ELSER W J THOMAS R A AND STEPLEY C I 1935 The desiccation of sera and other biological products (including microorganisms) in the frozen state with the preservation of the original qualities of products so treated Jour Immun 28 133-173
- EMILL WFFI I AND CALVIN I 1934 Contribution à l'étude des onychomycoses IV Onychomycoses causées par *Sterigmatocystis unguis* Arch Med Exp Anat Path Paris 28 463-465 illust
- ENGLER A AND PRANTL K 1907 Die natürlichen Pflanzenfamilien etc I Teil Abt 1 1-3
- FERDINANDSEN C C F AND WINGE O 1910 Fungi from Irof Warming's expedition to Venezuela and the West Indies Kob Bot Tids 30 208-222 1 *diptera* was one of a miscellaneous series described
- DA FONSECA OLYMPIO 1930 Mycetozoa por *Aspergillus amstelodami* Rev Med Cir Brasil 33 415-430 Pl 1 (1-19)
- FRAZER H C I AND CHAMBERS H S 1907 The morphology of 1 herbariorum Ann Mycol 5 419-431 Pls XI XII
- FREDEHLS G 1850-53 Beiträge zur Mykologie Frankfurt
- 1863 Beiträge zur Mycologie Heft 3 Frankfurt
- FILMER E I AND WEFAMAN C H 1930 An index to the chemical action of microorganisms on the non nitrogenous organic compounds 104 pp Springfield Illinois and Baltimore
- GALLOWAY L D 1930 The fungi causing mildew in cotton goods Jour Text Inst 21 T27-T286
- 1933 The stimulation by dilute antiseptics of sectoring in mold cultures (the action of salicylanilide on *A. terreus* in flour agar) Brit Mycol Soc Trans 18(11) 161-167
- GASTROCK F A PORCHER A WELLS P A AND MOYER A J 1938 Gluconic acid production on pilot plant scale Effect of variables on production by submerged mold growth Ind Eng Chem 30 782-789
- GILMAN J C AND ABBOTT E A 1927 A summary of the soil fungi In Sci Coll Jour of Sci 1 225-345
- GLISTER G A 1941 A new antibacterial agent produced by a mold Nature 163 410 1 *flatus*
- GOULD H S 1938 The metabolism of *Aspergillus tamarii* Kita. Kojic acid production Biochem Jour 32 97-89

- AND PAISTRICK H 1931 The biochemistry of microorganisms XL The crystalline pigments of species in the *Aspergillus glaucus* series *Biochem Jour* 25 1640-1646
- GREENE H C 1933 Variation in single spore cultures of *Aspergillus fischeri* *Mycologia* 25 111-138 3 figs 2 plates
- AND FRED F B 1931 Maintenance of vigorous mold stock cultures *Ind Eng Chem* 25 1797-98
- GUGLIELMI FERNAND 1901 Champignons Parasites d l'homme et des animaux Paris pp 462
- 1909 *Aspergillus fontinalis* nov sp parasite probable des nodosités juxta articulaires *Compt Rend Soc Biol* 66 10 7-103 also 67 10-17
- GUGLIELMI FERNAND 1911 Les souches sous-dermiques à répétition produits par l *Aspergillus fontinalis* n sp morphologique et biologique de cette espèce *Arch Parasitol* 14 11-137
- HALLER D ALBERTI 1747 Fauneraatio Microscopica stirpium Helvetiae Indigenarum etc
- 1768 Historia stirpium indigenarum Helveticae inchoata et
- HALLIER FRITZ 1870 Mitteilung über die Ohrpilze welche Herr Dr R Hagen in Leipzig zur mikroskopischen Untersuchung an Einsandte *Zeitschr Parasitenk* 2 200-231 Pls 5 and 6
- HANSEN H A 1938 The dual phenomenon in imperfect fungi *Mycologia* 31 417-455
- AND SMITH R E 1937 The mechanism of variation in imperfect fungi *Botrytis cinerea* *Phytopath* 27 953-961
- HANZAWA JICHI 1911 Untersuchungen über die Pilze auf dem getrockneten Boniten oder Katsunobu *Jour Coll Agr Tohoku Imp Univ* Dapporo 4 pt 4 p 215 Taf VII-VIII
- HENHARD P 1931 Polarité hérédité et variation chez diverses espèces de *Aspergillus* *La Cellule* XLIII 351-411 Pls 1-4
- VON HÖHNEL F 1907 Fragmente zur mykologie (II Mitteilung) *Sitzungsber K Akad Wiss Wien Math Naturh Kl* III 1 Abt p 97
- HOOPER I R ANDERSON H W SKELL P AND CARTER H E 1944 The identity of clavacin with patulin *Science* 99 16
- HOPPE A 1919 Ueber einen bisher unbekannten cellulose losenden im Verdauungstraktus vorkommenden *Aspergillus* *cellulosae* seine Zucht und seine Eigenschaften Centralbl f Bakt, etc 1 Orig 83 531-537
- HOPKINS S J AND CHIDWELL A C 1931 Growth of *Aspergillus versicolor* on higher parasitism *Biochem Jour* 26(1) 133-142
- HUBER G A 1933 *Aspergillus sclerotiorum* n sp and its relation to decay in apples *Phytopathology* 23 306-308
- JONES H RAKE G AND HAMRE D M 1943 Studies on *Aspergillus flavus* I Biological properties of crude and purified aspergillic acid *Jour Bact* 45 461-469
- KAROW E O 1942 The production of citric acid in submerged culture *Doctoral Thesis Rutgers Univ New Brunswick N J*
- KEITT O W 1915 Simple technique for isolating single spore strains of certain types of fungi *Phytopath* 5 206-209
- KINOSHITA K 1931a Über eine neue *Aspergillus* art *Aspergillus stactonicus* nov spec (Secondary title under the Japanese title) *Bot Mag Tokyo* 45(530) 45-61, figs 1-2 and Plate IV

- 1931b Über die Produktion von Itaconsäure und Mannit durch einen neuen Schimmelpilzen *Aspergillus itaconicus* Acta Phytochimica 5(3) 271-287 figs 5
- KITA, G 1913 Einige japonische Schimmelpilze Centralb f Baktr etc II Abt 37(17/21) 433-452 *A. tamaris* Kita
- KITA G 1914 Einige japonische Schimmelpilze II Mitt Über die Aspergillusarten aus Hatsuobushu und Vergleichung von vier *A. ochraceus* artigen Pilzen Centralb Baktr etc 2 Abt 41 351-363
- KNIPE II 1928 Die sexualität der niederen Pflanzen Differenzierung Verteilung Bestimmung und Vererbung des Geschlechts bei den Thallophyten Jena 1 *flavus* 1 *herbariorum* 1 *nidulans* *A. repens*
- KORSCHOLT O 1878 Ueber Sake das alkoholische Getränk der Japaner Dingler's Polytechnisches Journ 230 76-80 17-181 279-280 330-335, 471-427 *Eurotium oryzae*
- LAURENT E B 1939 A spore isolator combining some of the advantages of the LaRue and Hess methods Phytopath 29(3) 212-214
- LANGERON MAURICE 1922 Sur un champignon d'une otomycose brésilienne *Sterigmatocystis hortae* Bull Soc Path Exot 15 353-354
- LANGERON MAURICE 1922 Utilité de deux nouvelles coupures génériques dans les Heterosporiaceae *Diplomphanus* n gen et *Carpentella* n gen Compt Rend Soc Biol (Paris) 87 343 344
- LARUE C D 1920 Isolating single spores Bot Cas 70 319-320
- LINK H F 1800 Observationes in ordinibus plantarum naturales Gesellschaft Naturforschender Freunde zu Berlin Magazin 3 1809 this paper is usually cited as Link Obs
- 1824 Species Plantarum Ed 4 T 6 pt 1 p 65
- LINossier G 1901 Sur une hémative végétale l'aspergilline pigment des spores de l'*Aspergillus niger* Ann Microgr 3 309-32 also Compt Rend Acad Sci 112 489-497
- LOCKWOOD I B RAPER KENNETH B MOYER A J AND COOKILL R D The production and characterization of ultraviolet induced mutations in *Aspergillus terreus* III Biochemical characteristics of the mutations Am Jour Bot (in press)
- LUDWIG F 1832 Lehrbuch der Niederen Kryptogamen pp 2-8 Stuttgart
- MANGIN M L 1909 Qu'est ce que l'*Aspergillus glaucus*? Étude critique et expérimentale des formes groupées sous ce nom Ann des Sci Nat Bot Ser II 10 303-311 illus
- MARCHAL F 1923 Sur une espèce nouvelle du genre *Aspergillus* Mich *Aspergillus terricola* Revue Mycologique 15 no 69 101-103
- MARTIN G W 1911 Outline of the fungi Univ Ia Studies in Nat Hist VIII suppl pp 3-61
- MATTA A DA 1928 *Sterigmatocystis tropicalis* n sp de fungo pathogenico para o homem Bol Inst Brasil Sci 3 31-4 49
- MATTLET G 1921 Branchomyces dues à des *Sterigmatocystis* dans l'Urundi Communication préliminaire Ann Soc Belge Mic Trop 4 167 176 3 figs
- MICHEL C M AND MACBELLAMY H H 1913 An antibiotic substance produced by submerged cultivation of *Aspergillus flavus* Proc Soc Exp Biol and Med 63(2) 217-218
- MEHLICH A THLOG I AND FRED I B 1933 The *Aspergillus niger* method of measuring available potassium in soil Soil Science 35 2-9

- MEISSNER RICHARD 1937 Leber eine neue species von Eurotium *Aspergillus*
Bot Ztg 55 337-344 3.3-35" illus
- MICHELI PETRO A 1879 Nova plantarum genera 234 pp illus Florentiae
- MIRSKY H 1903 Sur quelques causes d'erreur dans la détermination les *Aspergillus* parasites de l'homme Thèse de Med Nancy 27 (1903) No 15
- MONTAGNE J F 1856 Sylloge generum specierumque Cryptogorum Paris
- MOSSERAT RAOUL 1931a Les *Aspergillus* de la section *Niger* Thom et Church
La Cellule tome XLIII fasc 2
- 1931b Sur la systématique des *Aspergillus* de la section *Niger* Thom et Church Ann Soc Sci Bruxelles 51 sér B 79
- MOYER A J WELLS P A STUBBS J J HERRICK H T AND MAY O E 1937 Gluconic acid production Development of inoculum and composition of fermentation solution for gluconic acid production by submerged mold growths under increased air pressure Ind Eng Chem 29 777-781
- NAKAWA R 1907 On the koji fungus *Aspergillus awamori* Inst of Govt Research Formosa Rept Vol I 1907 Vol II 1912 In Japanese
- SINO M AND WATANABE H 1936 Leber die garungorganismen in awamori Bereitung I *Aspergillus natus* Govt Research Inst Tawara Japan Rept no 177 (Reprinted from the Jour Agr Chem Soc Japan no 144)
- TAKEDA Y OKADA T AND SINO M 1931 Molds putrefying tobacco I Agr Chem Soc Japan Jour 10 135-197 illus (In Japanese Abstract in Chem Abs 23 8 1934)
- NEILL J C 1939 The mould fungi of New Zealand II The genus *Aspergillus* Roy Soc New Zeal Trans and Proc 69 237-264 illus
- OKASAKI K 1907 Eine neue *Aspergillus*art und ihre praktische Anwendung Centralb f Bakt abt 2 19 451-451
- 1914 Beiträge zur Affinität eines neuen weissen Fadenpilzes (*Aspergillus natus*) Centralb f Bakt Abt 2 42 225-240
- OTA M 1923 Sur une nouvelle espèce d'*Aspergillus* pathogène *Aspergillus jeanselmei* n sp Ann Parasit Humaine et Comp 1 137-146
- OWEN W L 1923 Mold fungi in sugar inversion Facts about Sugar 16 519-521 516-519
- OXFORD A E AND RAISTRICK H 194 Antibacterial substances from moulds IV Spinulosin and Fumigatin metabolic products of *Penicillium spinulosum* Thom and *Aspergillus fumigatus* Fresenius Chem and Ind 21 123
- PARTANBY ALEX M AND McPHERSON ROBERT R 1940 Testing mold resistant properties of oil paints Ind Eng Chem 32 443-445 illus
- PATOUILLARD M N 1891 Remarques sur l'organisation de quelques champignons exotiques II *Enricella varicolor* Berk et Br Bull Soc Myc Fr 7 45-47 pl 4
- PERQUIN L H C 1938 Bijdrage tot de kennis der Oxydatieve Dissimilatie van *Aspergillus niger* van Tieghem Thesis Delft
- PERSSON C H 1797 Tentamen dispositionis methodica fungorum
- 1801 Synopsis methodica fungorum
- PETRONEL H 1914 I germi atmosferici dei funghi con micelio Dissert Padova
- PHILPOT FLORA J 1943 A penicillin like substance from *Aspergillus giganteus* Wehm Nature 162 25
- PONTILLON CHARLES 1932 A physiological study of the lipids of *Sterigmatocystis nigra* Rev Gen Botan 44 411-49 465-83 56-60

- PRILL E A WENCK P R AND PETERSON W H 1935 The chemistry of mould tissue VI Factors influencing the amount and nature of the fat produced by *Aspergillus fischeri* Biochem Jour 29 21-33
- PRUESS L M EICHENGER F C AND PETERSON W H 1934 The chemistry of mold tissue II Composition of molds with special reference to the lipid content Centralbl Bakt Parasitenk u Infektionsk II Abt 88 310-377
- GORCICA H J GREENE H C AND PETERSON W H 1932 Wachstum und Steringehalt gewisser Schimmelpilze Biochem Zeits 248 401-413
- PETERSON W H AND FRED E B 1932 Isolation and identification of ergosterol and mannitol from *Aspergillus fischeri* Jour Biol Chem 97 483-489
- PETERSON W H STEENBOCK H AND FRED E B 1931 Sterol content and antirachitic activability of mold mycelia Jour Biol Chem 98 369-381
- QUEVEDO JOSÉ M 1912 Sur une encéphalomyélite épizootique d'origine mycétique chez les chevaux Premier Congr Internat Path Comp 2 200-209
- QUILICO A 1933 Sulla natura dei pigmenti delle spore dell *Aspergillus niger* Note III Sull *Aspergillina* Gazz Chim Ital 63(1) 400-410
- QUILICO A AND DICAPUA A 1933 Sopra i aspergillina i pigmenti delle spore dell *Aspergillus niger* Atti R Accad Lincei VI Rend Cl Sci Fis Mat e Nat 17 93-99
- RAISTRICK H ROBINSON ROBERT AND TODD A R 1937 The chemistry of *Aspergillus* colouring matters Part I (London) Chem Soc Jour 1937 80-88
- RAPER KENNETH B COGHILL R D AND HOLLANDER ALEXANDER The production and characterization of ultraviolet induced mutations in *Aspergillus terreus* II Cultural and morphological characteristics of the mutations Am Jour Bot (in press)
- RAPER KENNETH B AND THOM C 1944 New Species of *Aspergillus* from soil Mycologia 38 555-575 illust
- RAULIN J 1803 Etudes chimiques sur la végétation des Mucedinees Compt Rend Acad Sci (Paris) 57 228 also Ann Sci Nat Bot 5 ser II 93 1809
- RIDGWAY ROBERT 1912 Color Standards and Color Nomenclature 43 pp, illus Washington D C
- RIDLEY H N 1896 The Dracaenas of the Malay Peninsula Jour Bot (London) 34 152
- ROCKWELL G E AND O'FLAHERTY F 1931 Studies in the physiology of moulds II Composition and culture of molds Jour Amer Leather Chem Assoc 26 216
- RUFFOL, E 1937 Chemical composition of the fat from *Aspergillus citromyces* Jour Pharm Belg 19 63-8
- SABET LOUIS S 1939 On some fungi isolated from soil in Egypt Fouad I Univ Fac Sci Bull No 19
- SACCARDO P A 1891 Chromotaxia seu nomenclator colorum etc Patavii This gives 50 colors with a synonymy in Latin Italian French English and German This is cited as *Sacc Chromotaxia*
- SAITO K 1907 Mikrobiologische Studien über die Zubereitung des Bataaten brandtweines auf der Insel Hachijo (Japan) (*Asp batatae* A *pseudotarsus* n sp) Centralbl f Bakt Abt 2 18 30-37
- SAKAGUCHI K AND KODAMA K 1934 Classification of *Aspergillus* by means of the fermentation of sugars Jour Agr Chem Soc Japan 10 81-83
- SARTORY A 1913 Sur la présence d *Aspergillus fumigatus* Fr sur des cigares Compt Rend Soc Biol (Paris) 74 650-651

- 1920 Production de périthèces chez un *Aspergillus* sous l'influence d'une bactérie. *Compt Rend Soc Biol (Paris)* 83 1113-1114
- AND BAILLY A 1921 Du pouvoir agglutinant du sulfate de thorium sur les spores d *Aspergillus fumigatus* Fr. *Compt Rend Acad Sci (Paris)* 172 12-7
- AND FLAMENT L 1920 Étude morphologique et biologique d'un *Aspergillus* nouveau isolé d'expectorations d'un malade suspect de tuberculose pulmonaire. *Compt Rend Soc Biol* 93 1114-1115
- SARTORY R HUFFSCHMITT G AND MEYER J 1930 Un cas d'onychomycose provoquée par un *Furotium* nouveau *E. diplocyste* n. sp. *Compt Rend Soc Biol* 104 881-883
- SARTORY R AND MEYER J 1926 La formation des périthèces chez l'*Aspergillus fumigatus* Fresenius sous l'influence du radium. *Compt Rend Acad Sci* 183 130-2
- SARTORY R AND MEYER J 1927 Les variations des appareils végétatifs et conidiens de l'*Aspergillus fumigatus* Fresenius en cultures sur milieux dissociés et non dissociés sous l'influence des radiations du radium. *Bull des Sci Pharm* 34(4) 193-202
- SARTORY R AND MEYER J 1929 Un champignon nouveau de genre *Sterigmatocystis* (*Sterigmatocystis basidioparta* n. sp.) à basides cloisonnées. *Ann Mycol* 27(5-6) 317-320
- SARTORY R AND MEYER J 1930 Étude d'une nouvelle espèce de *Sterigmatocystis* *Sterigmatocystis albo rosea*. *Ann Mycol* 28(3/4) 358-359
- SARTORY R AND MEYER J 1930 Étude d'une nouvelle espèce de *Sterigmatocystis* *Sterigmatocystis cameleo*. *Ann Mycol* 28(3/4) 360-361
- SARTORY R AND MEYER J 1930 Étude d'une nouvelle espèce d'*Aspergillus* *Aspergillus halophilus*. *Ann Mycol* 28(3/4) 362-363
- SARTORY R AND MEYER J 1936 Effect of pressure or partial vacuum on the biochemical behavior of some lower fungi. *Compt Rend* 203 1280-91
- AND SYDOW H 1913 Étude biologique et morphologique d'un *Aspergillus* nouveau. *Ann Mycol* 11 no 2 ■ 156
- SCHIEHMANN ELISABETH 1912 Mutationen bei *Aspergillus niger* van Tieghem. *Ztschr Induk Abstam u Vererbungslehre* 8 heft 1/2 pp 1-35 fig 16 2 plates
- SCHMIDT C F (JR) 1935 The formation of fatty acids by *Aspergillus niger*. *Jour Bact* 30 445-46
- 1935 The formation of fatty acids from glucose by *Aspergillus niger*. *Jour Biol Chem* 110 511-20
- SCHWARTZ W 1928 Entwicklungsphysiologische Untersuchungen über die Gattungen *Aspergillus* und *Penicillium*. I *Aspergillus* Arten. *Flora* ■ 386-440
- SHIH Y K 1936 A taxonomic study of the genus *Aspergillus* around Wuchang Central China (Hyphomycetes). *Lingnan Sci Jour* 15(3) 36-3 8 15(4) 607-612
- SIEBENMANN F 1883 Die Fadenpilze *Aspergillus flavus niger fumigatus Eurotium repens* (und *Aspergillus glaucus*) und ihre Beziehung zur Otomyco- und aspergillina. *Zeitschr Ohrenheilk* 12 121-161
- SMITH GEORGE 1928 The identification of fungi causing mildew in cotton goods. The genus *Aspergillus*—Part I. *Jour Text Inst* 19 92-100

- 1931 The identification of fungi causing mildew in cotton goods The genus *Aspergillus*—Part II Textile Inst Jour 22 T110-T116 illus
- 1938 Introduction to Industrial Mycology pp 302 127 figs Edward Arnold London
- 1943 Two new species of *Aspergillus* Brit Mycol Soc Trans 25(1/2) 25-27 plates II and III *A. avenaceus* and 1 *proliferans*
- SPEAR A T 1912 Fungi parasitic upon insects injurious to sugar cane Honolulu Pap Sta Hawaiian Sug Pl Assn Path Physiol Sci Bull 12 1-6? *A. parasiticus*
- SPECIAZZINI C 1909 Fungi Argentini An del Mus Nac de Buenos Aires 6 81-365 Frequently cited as F Argent
- 1911 Mic Arg V An Mus Nac Buenos Aires, Ser 3 III 434
- SPIECKERMANN A AND BELMER W 1902 Untersuchungen über die Veränderungen von Futter und Nahrungsmitteln durch Mikroorganismen I Untersuchungen über die Veränderungen fettericher Futtermittel beim Schimmeln Landw Jahrb 31 81 128 illus
- STEINBERG ROBERT A AND THOM CHARLES 1940a Chemical induction of genetic changes in *Aspergilli* Jour Hered 31 61-63
- AND THOM CHARLES 1940b Mutations and reversions in reproductivity of *Aspergilli* with nitrite colchicine and d lysine Natl Acad Sci Proc 26 363-366
- STROMO F M AND PETERSON W H 1934 Chemistry of mold tissue IV The lipides of *Aspergillus sydowii* Jour Amer Chem Soc 58 957-958
- TAKAHASHI T AND YAMAMOTO T 1913 On the physiological differences of the varieties of *Aspergillus oryzae* employed in the three main industries of Japan namely aské shoyu and tamari manufacture Journ Coll Agr Tokyo 5 153-61
- TALICE R V AND MACHINOV J E 1931 *Aspergillus (Eurotium) montevideensis* n sp isolé d'un cas d'otomycose chez l'homme Soc de Biol (Paris) Compt Rend 108 1007-1009
- TAMIYA H AND MORITA H 1929-1930 Bibliographie von *Aspergillus* 1729 bis 1929 Bot Mag Tokyo Bd XLIII Heft 506 bis Bd XLIV Heft 524 Contains 2124 literature citations with index
- TALSON V O 1938 The conversion of energy by microorganisms V The accumulation of fat in *Aspergillus flavus* Microbiology (USSR) 7 300-5
- THOM C AND CHURCH M B 1918 *A. fumigatus* *A. nidulans* *A. terreus* n sp and their allies Amer Jour Bot 5 84-104
- AND CHURCH M B 1921 *Aspergillus flavus* 1 *oryzae* and associated species Amer Jour Bot 8 103-126
- AND CHURCH M B 1926 The *Aspergilli* pp 1 272 illus Baltimore
- AND CLARKE J A 1916 *Aspergillus niger* group Jour Agr Res 1 1 15
- AND LEFEVRE EDWIN 1921 Flora of corn meal Jour Agr Res 22 179-188
- AND RAPER KENNETH B 1939 The *Aspergillus nidulans* group Mycologia 31 653-669 illus
- AND RAPER K B 1941 The *Aspergillus glaucus* group U S Dept Agr Misc Pub 426 1-46 figures 14
- AND STEINBERG ROBERT A 1939 The chemical induction of genetic changes in fungi Natl Acad Sci Proc 25 329-333
- VAN TIEGHEM PH 1867 Recherches pour servir à l'histoire physiologiques des Mucédinées Fermentation gallique Ann Sci Nat Bot ser 5 8 210-211

- 1877 Sur la développement de quelques ascomycètes Bul Soc Bot France III 96-105 157-161, 203-206 206-210
- TIMONIN V I 1942 Another mould with antibacterial ability Science 96 491
- TIRABOSCHI C 1908 La proteolosi negli schizomiceti ifomiceti Giornale d. Soc de Igiene Milano *Aspergillus effusus*
- TRIZ H H 1914 The molds of cigars and their prevention U S Dept Agr Bul 109 1-8
- VAN DER BIL P A 1920 Studies on some fungi and the deterioration of sugar Union of South Africa Dept Agr Science Bul 17 31 pp. also Science Bul 18 19 pp
- VILLEMEN PAUL 1920 Nouvelles souches thermophiles d *Aspergillus glaucus* Soc Mycol de France Bul Trimest 26 177-136 illus
- 1922 *Sartorya* nouveau genre de Plectascinées angiocarpes Compt Rend Acad Sci (Paris) 184(3) 136-137
- WAKEMAN S A AND BLOIS ELIZABETH 1943 Strain specificity and production of antibiotic substances II *Aspergillus farus*-ory ac group Proc Natl Acad Sci 29 232
- HORNING E H AND SPENCER I L 1942a The production of two antibacterial substances fumigacin and clavacin Science 96 207-203
- HORNING ELIZABETH S AND SPENCER FREDERICK L 1942b Two antagonistic fungi *Aspergillus fumigatus* and *Aspergillus clavatus* and their antibiotic substances Jour Bact 45 233-245
- AND SCHWARTZ ALBERT 1943 Strain specificity and production of antibiotic substances Proc Nat Acad Sci 29 74-77
- WARD H E LOCKWOOD L B MAY O E AND HERRICK H T 1935 Production of fat from glucose by molds Cultivation of *Penicillium janczewicum* van Beyma in large scale laboratory apparatus 4 *farus* mycelium contained 16 percent fat Eng Chem 27 318-322
- WEAR P H W 1947 Studies on the elongation of the conidiophores of *Aspergillus giganteus* as affected by temperature nutrients and light Thesis George Washington Univ Washington D C
- WEHMER C 1891 Entstehung und physiologische Bedeutung der Oxalsäure in Stoffwechsel einiger Pilze Bot Ztg 49 233-246 and numerous additional pages in the same journal
- 1897 Zur Oxalsäurebildung durch *Aspergillus niger* Centralb f Bakt etc 2 Abt 3 102-108
- 1899 Ueber einige neue *Aspergillus* arten Bot Centralb 80 449-461 *A. varians* *A. minimus* *A. ostianus*
- 1901 Die Pilzgattung *Aspergillus* in morphologischer physiologischer und systematischer Beziehung Mémoires de la Société de Physique et d'Histoire Naturelle de Genève Tome XXXIII Seconde Partie pp 1-157 This reference is commonly cited as Wehmer's Monograph
- 1907 Zur Kenntnis einiger *Aspergillus* arten Centralb f Bakt etc III Abt Bd 18 385-395
- 1924 Oxalsäure und Zitronensäure Entstehung in ihrer gegenseitigen Beziehung bei verschiedenen Rassen des Pilzes *Aspergillus niger* Ber Deut Chem Gesell 57 1659-65
- WEISBERG P 1942 Bact ricidal effects of *Aspergillus clavatus* Nature 149 3-6-3-7
- WYCK P R GREENE H C AND FRED E B 1935 Factors influencing the growth and sterol formation of *Aspergillus fischeri* Jour Bact 29 89-90

- PETERSON W H AND FRED E B 1935 The chemistry of mold tissue IX Cultural factors influencing the growth and sterol production of *Aspergillus fischeri* Zentralbl Bakt Parasitenk u Infektionsk II Abt 92 330-338
- WHELDEN ROY M 1940 Mutations in *Aspergillus niger* bombarded by low voltage cathode rays Mycologia 32 630-643
- WHITE E C 1940 Bactericidal filtrates from a mold culture Science 92 127
- AND HILL J H 1943 Studies on antibacterial products formed by molds I Aspergillic acid a product of a strain of *Aspergillus flavus* Jour Bact 45 433-444
- WICKERHAM L J AND ANDREASEN A A 1942 The lyophil process its use in the preservation of yeasts Wallerstein Lab Comm 8(16) 165-169 1 plate
- WIGGERS FREDERICUS HENRICUS 1780 Primitiae Florae Holstinae 114 pp Kiliae (Facsimile ed 23 by W Junk 1975)
- WILHELM K A 1877 Beitrage zur Kenntniss der Pilzgattung Aspergillus Inaug Diss Strassburg pp 66
- WILKINS W H AND HARRIS G M C 1942 Investigation into the production of bacteriostatic substances by fungi I Preliminary examination of 100 fungal species Brit Jour Exptl Path 23 166
- WILSON J A AND DALB, G 1925 *Aspergillus niger*, a common mold that causes black spots on leather Jour Amer Leather Chemists Assoc n 20 400-405 figs 12
- WOLF FREDERICK A 1938 I Fungal flora of Yucatan caves Carnegie Inst Wash Pub No 491 p 19-21
- WOOLLEY D M STRONG F M PETERSON W H AND PRILL E A 1935 Chemistry of mold tissue X The phospholipides of *Aspergillus sydowii* Jour Am Chem Soc 57 2589-91
- YABUTA A 1912 On koji acid a new organic acid formed by *Aspergillus oryzae* Journ Coll Agr Tokyo 5 51-8 Orig Comm 8th Intern Congr Appl Chem (Append) 25 (1913) 45-62
- YAMAMOTO Y 1927 Ueber drei Stämme von *Aspergillus wentii* (Japanisch) Jour Soc Agr Forest Sapporo 19 123-40
- YUILL EDWARD 1939 Two new *Aspergillus* mutants Jour Bot (London) 174-175 illus
- AND YUILL JOHN L 1938 *Cladosporium olivaceum* a new Hyphomycete Brit Mycol Soc Trans 22 194-200 illus
- YUKAWA M 1911 Zwei neue *Aspergillus*arten aus Katsubushi Jour Coll Agr Tokyo 1 357-366
- ZIKES H 1922 Über die Perithezienbildung bei *A. oryzae* Centralbl f Bakt etc 2 Abt 56 339-343

CHAPTER XXIV

CHILCK LIST OF SPECIES AND GENERA

GENERIC NAMES FOUND APTIFIED TO ASPIERGILLI

Alliopsis G. Fim in Proc R I Acad 1853 and Jour Bot 1853 p 234 also Sac Fyll 18 No 8 16 1900 4 *Sapucaya* represents a monotypic genus found upon putrifying *Lecythis Sapucaya* From the description it was some member of the *A niger* group Saccardo's diagnosis is only seen

Ascopha *Ascophora nigrans* was vaguely assigned mostly to *Mucors* but appears to have been used by Raulin a group studying the tannic acid fermentation until Van Tieghem described *A niger*

Aspergillopsis Sopp in Videnskaps selskapets Skr I Nat Naturv kl No 11 pp 201 204 Taf XX fig 149 1912 Sopp probably had some members of the *A ustus* group

Aspergillopsis Spegazzini in An Mus Nat Buenos Aires Ser 3 III 434 1911 The black spored *Aspergilli* (essentially the *A niger* group) were regarded as dematiaceous and assigned to a new genus in that group Spegazzini's genus has not been accepted

Aspergillus Micheli in Nova Plantarum Genera p 212 Pl 91 1799 Compare Link in Obs p II 1809 and Corda in Icones Fungorum 4 III Tab XII fig 94 1840

Subgenus—*Microaspergillus* Wehmer in Monogr 1901 (Mémoires de la Société de Physique

et d'Histoire Naturelle de Genève Tome XXXIII Seconde Partie pp 1 15 1899-1901)

Subgenus—*Macroaspergillus* Wehmer in Monogr 1901 The subgenera are not accepted here

Cladosarum Vuill and Vuill in Trans Brit Myc Soc 22 194-200 Its 11-13 1934 Regarded as a laboratory mutant not encountered in nature In *A niger* group See

Dimargaris van Tieghem in Ann Sci Nat 6 Ser 1 54 18 5 The name *Dimargaris* sp has been seen upon living cultures in one of the great laboratory collections These cultures were obtained from dog and squirrel dung The organism so labeled belonged to the *Aspergillus candidus* group The genus *Dimargaris* (type *D crystalli* gena v Tieghem) is regarded by Fitzpatrick as closely related to *Dispira* and as a synonym of *Dispira* by Zycha (Kryptogamenflora der Mark Brandenburg Pilze II Mucorineae Band VIa 1 164 114 figs 1935) In any case it is only reported as a parasite of the mycelia of the Mucorineae hence certainly was not an *Aspergillus*

Diplostephanus Langeron in Compt Rend Soc Biol Paris III 343-345 1900 This genus is proposed by Langeron to include ascosporic *Aspergilli* (*Sterigmatocystis*) with two series of

- sterigmata *A nidulans* Eidam
 ■ named as type The proposal
 ■ rejected here 8
- Emericella* Berkeley and Broome in
 Introd Crypt Bot pp 340-341
 fig 76 1857 See also Pa-
 touillard in Bul Soc Myc
 France 7 43-49 pl 4 figs 6-12
 1891 Based upon *E varicolor*
 Syn *A varicolor* q v 163
- Euaspergillus* Ludwig in Lehrbuch
 der niederen Kryptogamen p
 258 Stuttgart 1892 The
 genus was proposed to cover the
 sclerotium producing *Aspergilli*
 such as *A niger* *A flavus* *A*
ochraceus The name has not
 been accepted 8
- Eurotium* Link in Obs p 31 Taf 2
 fig 44 1809 Link described
Aspergillus glaucus ■ a conidial
 mold and *Eurotium herbariorum*
 for the ascospore form suppos-
 ing them to be different fungi
 His genus *Eurotium* has been
 widely accepted but it is re-
 jected here for reasons discussed
 elsewhere Forms listed as
Eurotium but having by descrip-
 tion black perithecia are
 excluded No species with
 aspergilloid conidial structures
 has black (Dematiaceous) peri-
 thecia 7
- In *engata* Borzi in Jahrb Wiss Bot
 (Lipsheim) III 450-463 Pl
 19 20 (1884) 1885 Type sp
I erythrospora Borzi Syn
A varicolor q v 165
- Mucor* used by Wiggers (Wichers)
 in *Immities Florae Holatiae*
 1780 (Republished in facsimile
 Edition No 23 by W Junk in
 1925) *Mucor herbariorum*
sessilis luteus is given as No
 1158 7
- Rhodocephalus* Corda in Icones I
 21 1839 and the figures given for
R aureus in Icones III Taf
 II fig 33 suggest *A terreus*
- Sartorya* Willemin in Compt Rend
 Acad Sci (Paris) 184 136-317
 1927 Inadequately described
 as an ascospore phase of *A*
fumigatus It was not distribu-
 ted in culture 9
- Sterigmatocystis* Cramer in Viertel
 jahresschrift der Naturforschun-
 gen Gesellschaft Zurich 4 325
 1859 Type *A anteaustica*
 Cramer Cramer proposed to
 include in his new genus all
 aspergilloid species showing
 both primary and secondary
 sterigmata *S niger* (*A niger*)
 was the organism under discus-
 sion hence the type species
 Many mycologists have agreed
 with Cramer others such as
 Fischer Wehmer and Thom
 have rejected *Sterigmato-*
cystis 8
- ### CHECK LIST OF NAMED SPECIES
- S acinus* uae Caballero in Boletín
 R Soc Esp Hist Nat ■ 470
 1924 229
 Syn *A carbonarius* fide Blochwitz
 or *A pulchellus* fide Mos-
 seray 229
- S aerea* Bainier in Bul Soc Bot
 France 28 78 1891 *A ventii*
 group 249
- A africanus* Dur et V in Fl Alg
 IP 342 1849 Not an *Asper-*
gillus 7
- A agens* This name is cited by
 Lindt in Arch Exp Path
 Pharm 25 263 1889 as taken
 from Saccardo's *Myloge-*
 search for this reference leads to
 the conclusion that in this cita-
 tion *A Hageni* was made to read
A agens
- S alba* Bainier in Bul Soc Bot
 France 27 30 1880 Same
 member of *A candidus* group 211
- S alba cyanogena* Bourge listed
 among cultures in the Bourge
 Collection 1939
- S alba lutea* Bourge n m in M^h
 p 9 among the *luteus* series

- A. alba roseus* attached to a culture in the Bannier Collection and by inference in the Biourge Collection as near *A. nircus* Blochwitz Thom No 4640 490
- S. alba sclerotifera* Biourge nomen nudum listed in his MS as related to *A. okanaki*. It produces a violet or blue color in reverse
- S. alba sulphurea* Biourge nomen nudum listed in MS as near *A. okanaki*. It produces a blue or violet reverse
- A. albidus* Eichelbaum in Verhandlungen d Naturw Ver Hamburg 3 Folge IV 35 1906
Syn *E. albidum* Sacc Sill 22 1:54 1913
- A. albidus* Speg. A culture under this label distributed by Biourge represents a strain of *A. nircus glaucus* q v 135
- S. albo lutea* Bannier in Bull Soc Bot France 27 30 1880. This was a small pale yellow form but no further data were given and it has not since been identified. Apparently close to *A. carneus*
- S. albo lutea* Sartory Sartory and Meyer cited by Blochwitz in Ann Mycol III 3 1933. Some member of the *A. candidus* group 211
- A. albo marginatus* Biourge in MS III ? as change of name from *Pencilium albo marginatum* figured only in the Pencilium Monograph in La Cellule tome XXXIII 1 fasc fig 129. It was some member of the *A. restrictus* series received and studied in Thom No 4733 134a. Near *A. gracilis* in the *A. glaucus* group 139
- S. albo osea* Sartory Sartory and Meyer in Ann Mycol 28 358-359 Pl III figs 1-6 1930. Compare *S. albo rosea* in Blochwitz Ann Mycol 31 7 1933. *A. carneus* series 202
- A. albus* Wilhelm Inaug Diss Strassburg # 69 1877. Probably *A. albus* Haller. *S. alba* Sacc. *Monilia alba* Persoon. In *A. candidus* group 211
- A. alliaceus* Thom and Church. The Aspergilli p 163 1926. See *A. wentii* group 244
- A. alternatus* Berkeley in Ann Nat Hist Ser 1 Vol 1 202 1838. Not an Aspergillus
- A. alutaceus* B and C in Grevillea 3 No 25 p 108 1875. In the *A. ochraceus* group unidentified. fable to strain or series 281
- S. ambaris* (alternative spelling *ambaris*) Beauregard in Ann de Micrographie 10 255-278 1 plate 1899. Cited without description in Compt Rend Hebdom des Séances de la Soc de Biol 28 Mai (1898). *A. vesiculor* group 192
- A. amoenus* Roburg in Hedwigia 70 138-9 1930. Distributed by the Centraal bureau voor Schimmelcultures Baarn. Received by us on November 20 1933 and determined as a member of the *A. gracilis* series but the description allies it with *A. versicolor*
- A. amstelodami* (Mangin) Thom and Church in The Aspergilli p 113 1926 also Thom and Raper U S D A Misc Publ 476 pp 22-26 1941 172
- Syn *E. amstelodami* Mangin in Sci Nat Bot Ser 9 10 360-361 1909. *A. glaucus* group 122
- Incorrectly spelled *A. amsterodami* in Nakazawa Jour Agr Chem Soc Japan 10(2) 1934
- Syn *E. repens* var *amstelodami* Vuill in Soc Mycol de France Bul Trimest 36 131 1920
- var *alophote* Duché cited by Dodge in his Med Mycol p 630 1935. Ascospore lacks the crest of the species

- A. anomalus* Mosseray in La Cellule \LIII 248-249 pl 4 figs 107-112 1934 Member of *A. niger* group 234
- S. anilacustica* Cramer in Vrtlschr Naturf Gesell Zürich Jahrg 4 Heft 4 325 1859 In *A. niger* group 239
Syn *A. phoenixis* in *A. niger* group 222
- A. archaeoflavus* Blochwitz in Ann Mycol 31(1/2) 73-83 1933 The type culture (Thom No 250-5346) was too close to *A. wentii* for satisfactory separation 247
- A. archiflavipes* Blochwitz in Ann Mycol 32(1/2) III 1934 A form of *A. flavipes* characterized by deep brown to red shades of color in the mycelium 181
- A. argentinus* Spegazzini in Rev Agr Veter LaPlata p 245 1896 A conidial form in the *A. glaucus* group not identifiable
- A. argillaceus* was distributed but not described by Biourge It appears to be a non ascospore strain of the *A. repens* series 111
- A. atrofuscus* Mosseray in La Cellule \LIII 266-270 pl 4 figs 126-129 1934 Member of *A. niger* group 235
- A. atropurpureus* Zimmermann in Centbl Bakt [etc] Abt 2 Bd 8 No 5 p 218 1902 *A. niger* group 226
- A. atropurpureus* Blochwitz in Ann Mycol 32(1/2) 86 1934 Blochwitz proposed this name to cover purple brown series in *A. niger* group 225
- A. atro ruber* Estienne
Syn *Penicillium roseo-cinnabarinum* Biourge in La Cellule 33 fasc 11 319-321 Pl \VII fig 97 1923
Syn *Physomyces heterosporus* Harz fide Biourge
Syn *S. atro rubra* (Estienne) Biourge Mb
- A. atro violaceus* Mosseray in La Cellule \LIII 268 269 pl 4 figs 122-125 1934 235
- Blochwitz in Ann Mycol III 240 1934 identifies this with *A. violaceo fuscus* Gasperini 231
- A. atrovirens* Karst in Symb 26 78 Sacc Syl 10 524 Inadequately described for identification
- A. aurantiacus* Berkeley in British Fungi fasc IV 1843 Cited by Montagne in Ann Sci Nat Bot 3 Ser 12 299 1849
Syn *Nematogonium aurantiacum*
- S. aurea* Greco in Origine des Tumeurs et Mycoses Argentines Buenos Aires pp 641-694 figs 418-428 1916 Not since recognized Some *A. ochraceus* strain
- A. aureoglauca* Roburg in Hedwigia 70 137 1930 Culture some strain of *A. repens* contributed by Roburg Noted as some member of *A. glaucus* group by Blochwitz in Ann Mycol 33 240 1935
- A. aureus* Berkeley in English Flora 5 316 1836 The golden yellow elliptical conidia reported by Berkeley suggest *A. citrisporus* but actual identification from the description impossible 719
- A. aureus* Nakazawa in Inst Govt Res Formosa Rept Vol 1 1907 719
- A. aureus* varieties described by Nakazawa Sano and Watanabe in Jour Agr Chem Soc Japan No 144 1936 (In Japanese)
- var *acidus* 720
var *brevis* 720
var *minor* 720
var *murinus* 720
var *pallidus* 720
- comparison of figures and measurements given convince the authors that these varieties could not be safely identified by descriptive data

S. auricoma Guéguen in Bull Soc
Mycol France 15 11 187 figs
1-49 1899 In 1 ochraceous
group

4 *auricularis* Moquin Tandon in
Flements Bot Med 2ed p 466
1866 This name was not
accompanied by adequate de-
scription to separate the form
intended from other species
which are occasionally found in
the ear

1 *ariarius* Peck in N Y State
Mus Rept (1890) 41 120
1897 A strain of *A. fumigatus*
isolated from a canary

4 *arenacea* George Smith in Brit
Mycol Soc Trans 25 21 27
Pl 1 figs 13 1913

1 *avomori* Nakazawa in Inst of
Govt Research Formosa Rept
1 1907 and 2 1912 A member
of the *niger* group

A *avomori* varieties described by
Nakazawa Simo and Watanabe
in Jour Agr Chem Soc Japan
No 141 1936 (In Japanese)

var *fe rugineus*

var *fumeus*

var *fuscus*

var *minimus*

var *piceus*

No doubt the describers had a
series of industrially significant
strains but it is doubtful if others
could identify them from the
description and figures given

4 *avomori* Usamu in Myk Cen-
tralbl 4 193 1914 Species
recognized by Mosseray in La
Cellule 43 264 1931 With *A.*
avomori Nakazawa cited as a
synonym

A *bainieri* Mosseray in Ann Soc
Sc Bruxelles 54 ser B 79
1934

Syn *A. longobasidia* Bainier fide
Mosseray in La Cellule XLIII
(2) 277 1934

A *barbæ* Castellani Cited by
Sartory A in Champignons

parasites de l'homme et des
animaux p 205 1922 This
organism was found in the beard
of a native of Uganda and again
from Ceylon The only further
information given is conidia 4 to
5 μ globose dark brown

S. basidiosepta Sartory and
Meyer in Ann Mycol 27 317-
320 Pl 11 1929 A variant
of the *A. candidus* group

A *batatae* Saito in Centralbl f Bakt
2 11 18(1/2) 34 1907 In
the *A. niger* group

1 *belantii* Carbone in Atti d Inst
Bot Univ Iavia Serie II Vol
XIV 63 fig 11 1914 Prob-
ably in the *glauca* group

1 (Sporodinia) *bellomontii* Mon-
tagne in Ann Sci Nat Bot Ser
4 12 181 182 1850 Noted
as related to *A. maximus* (Sporo-
dina) Not an *A. pergilii*

S. bicolor J Ray in Rev Sc Ser 4
1 8 176-177 193 1912 Lille
189 Probably in the *aydonii*
series

1 *bourgei* Mosseray in La Cellule
XLIII 211 212 pl 4 figs 81-
83 1934 One of the *A. niger*
group

S. blanc jaune Baumer nomen
nudum A culture so labeled
from the Baumer Collection rep-
resents a diminutive member
of the *A. candidus* group

1 *blochwitzii* Biourg nomen
nudum listed in Biourg MS
(p 3) among the *A. claratus*
group as a synonym of *A. cla-
ratus* var *gigantea* Blochwitz
A. boedijnii Blochwitz in Ann
Mycol 32 83-89 1934

Syn *A. tercus* var *boedijnii*
E. bonariense Peg in Anal de la
Soc Cient Argen 10 171
1880 Some member of the *A.*
glauca group but data are
inadequate

Coenium Bozianum Saccardo
See *A. varicolor*

- A. bouffardi* Brumpt (1905) Cited by Castellani and Chalmers in *Man Trop Dis* p 805 1913
Syn *Madurella Bouffardi* (Brumpt) Dodge Med Myc p 685 1935
- A. Brodens* (Mattlet) Dodge in Dodge Med Mycol p 635 1935
Syn *S. Brodens* Mattlet in Ann Soc Belge Med Trop 4 167-171 figs 1 2 1924 Probably *A. unguis*
var *Lancampenhouti* (Mattlet) Dodge in Dodge Med Myc 636 1935
- A. bronchialis* Blumentritt in Ber Deut Bot Ges III 442-446 pl 22 figs 1-6 1901 also ibid 23 419-427 pl 19 figs 1 3 6 7 8 19 and 20 1905 In the *A. fumigatus* group 151
- A. brunneo fuscus* See in Les Malades du papier piqué Paris p 29 1919 Unidentified except as a member of the *A. glaucus* group
- A. brunneo virens* Delacroix in Bul Soc Myc France III 120 with text figure 1897 Unidentified except as a member of the *A. glaucus* group
- A. brunneus* Delacroix in Soc Mycol Bul France 9 185 Pl XI fig III 1893 Delacroix described this as the conical stage of *A. echinulatus* q v 131
- S. Buntingiana* Biourge nomen nudum listed in Biourge's MS as applied to a culture of a member of the *A. candidus* group received from Bunting
- A. Buntingii* Mosseray in La Cellule XLIII 236-238 pl 4 fig 91-95 1934 Member of *A. niger* group in Biourge Collection 1939 233
- A. butyracea* (Bainier) n. comb 282
Syn *S. butyracea* Bainier in Bull Soc Bot France 27 29 1880
C Roumeguere's Fungi Gallici Exsiccati No 93 is recorded as Bainier's material In the *A. ochraceus* group 282
- A. byssoides* Sprengel in Sys Veg ed 16 V 4 541 1827 A fungus on rotting paper with globose fuscous heads but not identifiable
- A. cacao* This name appeared upon a culture in the Bainier collection (Thom No 4640.397) and has been contributed also by Pribram (4777 4) The organism is a strain of *A. lanarius* 2,6
- A. caesiellus* Saito in Jour Coll Sci Imp Univ Tokyo III 49 Pl III fig 14 1904 Regarded by Neill (in Royal Soc New Zealand Trans III 242 1939) as *A. restrictus* G Smuth 141
- A. caespitosus* n. sp. Raper & Thom in Mycologia 36 563-565 fig 3 1944 A member of *A. nidulans* group 166
- A. calyptratus* Oudemans in Arch Neerl II 7 283 pl 13 1902 See *A. fumigatus* group 151
var *italicus* Ferraris in Ann Mycol 10 294 1912
- S. cameleo* Sartory Sartory and Meyer in Ann Mycol 29 360-361 Pl III 1930 Some variant of *A. sydowii* 186
- S. candida* Sacc in Michelia 1 p 91 1877 In the *A. candidus* group
- S. candidula* Bainier in Sacc Syll 4 73 1886
Syn *S. candida* Bainier in Bull Soc Bot France 27 30 1880 A member of the *A. candidus* group
- A. candidus* Lank in Obs p 18 1809 207
var *thermophilus* Nakazawa et al in Jour Agr Chem Soc Japan 88 17 1932 (in Japanese) cited Blochwitz Ann Mycol 33 244 1933
- A. capitulo pullo* Haller in Historia Stirpium Indigenarum Helvetiae

- Incosta* etc 1768 Appar
 ently taken from Michel 1729
S. carbonaria Baimier in Bul Soc
 Bot France 27 2-93 1850
 See 1 *carbonarius* (Baimier)
 Thom In 1 *niger* group 279
A. carbonarius (Baimier) Thom in
 Jour Agr Res 7 17 1916 279
A. carbonarius seu *ate* Meis and
 Larascandalo in Gaz Ospedali
 18 69-77 1895 Cited by
 Dodge in Med Myc 6 9 as not
 an *Aspergillus*
A. cornutus Sacc in Michelia 1 p
 77 1577 And Fungi italici
 No 18 In *A. glaucus* series
A. carneus (van Tieghem) Bloch
 witz in Ann Mycol 31(1/2)
 81 1933
 Syn *Sterigmatocystis carneus* van
 Tieghem in Bull Soc Bot
 France 24 103 18 7 See also
 Saccardo Sylloge 4 4 and
 Wehmer's Monograph (Mem
 Soc Phys His Nat Gen pp
 1 187) 1899-1901 Species in
A. teus group
 var *subglobosa* Blochwitz in Ann
 Mycol 33 243 1935
 var *opaca* Blochwitz in Ann
 Mycol 33 219 1935
A. carnyi Biourge deser Thom and
 Raper in U S Dept Agr Misc
 Pub 476 p 31-5 1941
S. castagnei Biourge undescribed
 culture in the Biourge Collec
 tion listed as near *A. carbo*
naeus in the *A. niger* group
A. castanea Patterson in Bul Torrey
 Bot Club 27 284 1900 In *A.*
tamaris series
A. cellulosa Hlopffe in Centralb f
 Bakt etc 1 abt 83 531-37
 1919
 Syn *A. fumigatus*
A. cernuus Massee in Ken Misc
 Bul 4 158 1914 Neill con
 sidera *A. gratiosi* Sartory to be a
 synonym but that is not
 probable
 1 *chevalieri* (Mangin) Thom and
 Church in The *Aspergilli* p
 111 1906
 Syn *L. chevalieri* Mangin in Ann
 Sci Nat Bot Ser 9 10 361-
 367 fg 12 1909 In the 1
glaucus group
A. chevalieri var *intermedius* Thom
 and Raper in U S Dept Agr
 Misc Pub 476 p 21 fg 8B
 1911
A. chevalieri var *multiascosporus* 191
 Nakazawa Takada Okada and
 Sino in Agr Chem Soc Japan
 Jour 10 135-197 1934 Re
 ceived from Hsarn and appears
 as NRRL No 88 A strain of
 1 *chevalieri*
F. chilense Montagne in Syll Crypt
 No 919 Fl Chil VII p 4 6
 cited by Saccardo Syll 1 p 27
 In the *A. glaucus* group
S. chloina Cooke and Massoe in
 Greville 18 7 1889 Probab
 ly a nonascosporic strain of the
A. glaucus group
A. chysospermum Thaxter nomen
 nudum attached to a culture
 later shown to be *A. citrisporus*
 Van Hohenel q v
A. chungii Shih in Lingnan Sci
 Jour 18(3) 378 1933 In the
A. flavus group
A. churchii Vosserey in La Cellule
 XLIII 247 244 pl 4 figs 76-80
 1934 Representative of the *A.*
niger group In the Biourge
 Collection 1939 234
A. cinnmeus Berkeley and Curtis
 in Greville 3 108 1875 This
 specimen was identified by Dr
 Farlow (Bibliographical Index
 I pt 1 page 277) as *Periconia*
chlorocephala Fres
A. cinerescens Baimier and Sartory
 in Bul Soc Mycol France 27
 99-101 pl III figs 6-12 1911
 In the 1 *glaucus* group
A. cinctus Spegazzini in Ann Soc
 Cient Argen 10 162-163 1880

- A. disjunctus* Baimier et Sartory in Bul Soc Myc France 27 346-368 pl V-VI 1911 A member of the *A. glaucus* group See *A. echinulatus* 133
- 1 *diversicolor* Waksman in Soil Science 2 126 1916 The reference to an organism by Waksman under this name was a typographical error for *A. versicolor*
- S. dubia* (B & Br) Sacc in Fungi italici pl 902 and Sylloge 4 72 1886
- Syn *A. dubius* Corda in Berkeley and Broome in Ann Nat Hist 2 Ser 7 100 (No 590) 1851 Not thus far recognized
- A. dubiosus* Lindau in Rabh krypt Fl 8 151 1907 In the *A. candidus* group
- A. eburneus* Biourge nomen nudum was attached to a culture received from Biourge and recorded as NRRL 515 It is accepted as *A. niteus* 702
- A. echinosporus* Sorokin Abst by Busch in Ztschr f Pflanzenkrankheiten 3 155 1893 Ref in Sacc Sylloge 11 592 1905 The description suggests a *Ilaplographium*
- 1 *echinulatus* (Delaer) Thom and Church in The Aspergilli p 107 1920 131
- Syn *E. echinulatum* Delaer Soc Mycol de France Bul Trimest 9 266 Pl XIV fig III 1893
- Syn *E. verruculosum* Vuill in Soc Mycol de France Bul Trimest III 83 1918 In *A. glaucus* group 131
- A. effusus* Tiraboschi in Ann di Bot 7 (fasc 1) 16 1908 See also Thom and Church in Am Jour Bot 2 109-110 1911 Described originally from rotten corn (*Les Mays*) belongs to the *A. flatus-oryzae* group 767
- A. elatior* Mosseray in La Cellule VI III 253 255 pl 3 figs 29-32 1931 In the Biourge Collection 234
- A. elegans* Casperini in Atti Soc Toscana Sci Nat Pisa Mem 8 (fasc 2) 325 188 In *A. ochraceus* group 251
- E. epizylon* Kunze and Schm (D Schw No 88 Wallr Fl Crypt No 2078) In Compendium Florae Germanicae 4 331 1883 This was evidently some member of the 1 *glaucus* group
- A. erythrocephalus* Berkeley and Curtis in Jour Inn Soc [London] Bot 10 367 1869 In *A. tamaris* group 257
- Inzengaea erythrospora* Borzi in Jahrb Wiss Bot (Pringsheim) 16 450-463, pls 10-20 1881 Manifestly *A. varicolor* 163
- 1 *exiguus* Hann
- Syn 1 *conicus* fide Blochwitz in Ann Mycol 31 73 1933
- S. ferruginea* Cooke in Grevillea VIII (1879) 95 in Jour Quekett Mic Club 2 Ser 2 139 1835 Not an *Aspergillus* Re published in Veg Wasps and Plant Worms p 184 1897 see also Petch in Trans Brit Myc Soc XVI p 72 1931 who examined type specimens as rust colored patches on pupae conidophores hyaline smooth 12μ diam vesicle globose 50μ primary sterigmata 30 by 4 to 12μ secondary 14 18 by 7 9μ conidia from 9 by 5μ to 11 to 9μ subglobose brown coarsely rough a brown member of the *A. niger* group
- A. ferrugineus* Fuckel in Fungi rhenani No 157 also Symbolae Mycologicae in Jahr d Nassauischen Vereins fur Naturkunde Jahrg XVIII and XXV 3-8 1869-70 Not identified
- A. ferrugineus* Link in Sp Plant Ed 4 6 pt 1 p 68 1821 Also in Fries Sys Myc 3 347 1879 Both authors seem to have had members of the *A. glaucus* group
- A. ficuum* (Reich) Hennings 725

- Syn *Sterigmatocystis fuscum* (Reich) I Henn in Hedwigia 31 Heft 2 86 1893
- Syn *Ustilago fuscum* Reichardt in Verhandl K K Zool Bot Gesell Wien 17 33 1867 A culture distributed under this name (Thom 14?) is a rapid oxalic acid producing organism In the *A niger* group
- A fementis* Sopp cited as a name on a culture by Biourge in M^s p 19 in the *A flavus* group
- A fimelarius* Peck in N Y State Mus Bot Rept 4? p 128 1889 Probably 1 *candidus* group
- A fimelis* Sacc in Michelia 2 343 189? Inadequately described
- A fischeri* Wehmer in Centralb f Bakt II Abt 18(12/15) 390-39? figs 5 1907 In the 1 *fimigatus* group 151
- Syn *A fumigatus-ascosporic* see Thom and Church in Am Jour Bot 8 91-9? 1918
- A flavo-ous* Biourge nomen nudum listed in his M^s as a member of the 1 *flavus-oryzæ* group with yellow-orange reverse as shown by a culture in his collection
- A flavescens* Wrethen in Compt Rend Acad Sci Paris III 368 188 Also St Petersburg Med Zeitschr II 133 1867 Identifications have not been satisfactory Possibly in 1 *fulvulus* group
- A flavus* Berkeley and Broome in Jour Linnean Soc (London) Bot 14 101 1845 Cited by Saccardo in Fungi of Ceylon London 1871-3 No 913 b Probably a member of the 1 *flavus* group but not more closely identifiable
- A flavipes* (Bainier and Sartory) Thom and Church in The Aspergilli 155 1916 19
- Syn *S flavipes* Bainier and Sartory in Bul Soc Mycol France 27 90-96 pl III figs 1-6 1911 179
- 1 *flavoviridescens* Hanzawa in Journ Coll Agr Tohoku Imp Univ Sapporo 4 23? 3 11 21 figs 1-4 1911 In the *viricolor* group 10?
- A flavus* Link in Obs p 16 1809 (H F Link Observations in Ordines plantarum naturales Gesellschaft Naturforschender Freunde zu Berlin Magazin 3 1809—commonly cited Link Obs) 263
- A flavus* forma *Maydis* Ciferri R in Ann Mycol 20 46 1922
- A flavus* var *japonica* cited by Blochwitz in Ann Mycol 31 74 1933 without description
- A flavus* var *viridis* Blochwitz in Ann Mycol 31 86 1934 Is vaguely designated as a non tropical strain apparently duplicating 1 *parasiticus* Speare 267
- 1 *flavus* mut *rufa* Blochwitz in Ann Mycol 27(3,4) 201 1929 A mutation which shows gradations to pure brown from the green form
- A flavus* mut *fusca* Blochwitz in Ber d Bot Ges 48 56 1928
- Eurotium aspergillus flavus* DeBary and Wor Terminology employed by DeBary and Woronin (Biot s Morph u Physiol d Pilze III Reihe p 380 1890) to designate relationship of the asexual species *A flavus* with species characterized by a perfect stage and included in *Eurotium* 263
- A foetidus* n sp 219
- Syn 1 *aureus* Nakazawa A black *Aspergillus* with a strong musty odor 219
- A fonscaneus* n sp
- Syn *S fusca* Bainier in Bul Soc Mycol France 27 29 pl 1 fig 5 1880 A black *Aspergillus* on

- Session Tunis pp 601-603
1913 Some one of the 4 *glau-
cus* group 133
- A *gracilis* Bainier in Bul Soc My-
col France 23 92 Pl I figs
11-14 1907 In 4 *restrictus*
series 138
- var *exiguus* Bainier & Sartory in
Bull Soc Mycol France 28 47
pl III 1912 According to the
description this variety differs in
physiological characters slightly
from 1 *gracilis* Bainier 140
- 1 *granulatus* Mosseray in La Cel-
lule LIII 219-50 pl 3 figs 25-
28 1934 A member of the 1
niger group in the Biourge Col-
lection 234
- A *granulosus* Raper and Thom in
Mycologia 36 565-568 fig 4
1944 In the *A ustus* group 145
- A *gratioli* Sartory in Compt Rend
Acad Sci (Paris) 170 523-524
1920 Also in Champignons
parasites de l'homme et des
animaux pp 578-579 1922
Probably same member of the *A*
fumigatus group 154
- A *Greonis* Dodge in Dodge Med
Myc p 634 1935
- Syn *S aurea* Greco q v
- A *griseus* Link in Sp Plant Ed 4
6(1) 69 1824 Incorrectly cited
by Bonorden and Wehmer as
Link Obs 1 69 1809 Name
also used by Fries and by Bonor-
den Not identified
- A *guegueni* was figured in Biourge's
monograph of the *Penicillia* (La
Cellule t 33 fasc 1 pp 7-330
1923) as *P guegueni* Plate XX
He afterward distributed it as 1
guegueni In *A restrictus* series 139
- 1 *guttifer* Mosseray in La Cellule
LIII 235-236 pl III fig 53-
57 1934 In the Biourge Col-
lection 233
- 4 *gymnosardae* Yukawa in Jour
Coll Agr Tokyo I 362 Pl II
figs 1-7 1911 This fungus
was found by Yukawa under the
name *avolabi* and is de-
scribed by him as essential to the
ripening of the tunafish prepara-
tion katsuobushi The di-
mensions given are intermediate
between those of 4 *flavus* and *A*
oryae and closely approximate
those of 1 *pseudo flatus* A
though we have cultures related
to these forms we have not been
able to identify these intermedi-
ates except as members of the *A*
flatus-oryae group 260
- A *hagens* Hallier in Cattaneo Mico-
Corp Um II 123 Pl 6 fig 8
Florentin 1892 146
- Syn *Otomyces Hagens* Hallier in
Zeitschr Parasit 1 195 1869
2 22 233 and 249 Pl 5 1870
Not identifiable
- A *halophilus* Sartory Sartory and
Meyer in Ann Mycol XXXIII
362-363 Pl III figs 11-14
1930 Probably some nonasco-
sporic member of the *A glaucus*
group 118
- 1 *helicophorus* nomen nudum at-
tached by Thaxter to a culture
that was found to belong with 1
ustus 174
- S *helia* Bainier in Bul Soc Bot
France III 78 1881 In *A*
ochraceus group 291
- A *hennebergi* Blochwitz in Ann
Mycol 33 238 1935 Regarded
by Veill as one of the 1 *ochra-
ceus* group See 1 *ventii* group 249
- A *herbariorum* species name seems
to appear first as *Mucor herbario-
rum* in Primitiae Florae Holste-
ticae by Fredericus Henricus
Wiggers 1750 republished in
Facsimile edition No III by W
Junk 1925 See 1 *glaucus*
group 100
- Eurotium herbariorum* ser minor
Mangin in Ann des Sci Nat
Bot (ser 9) 10 36 1909 See
1 *mangini* 127
- Eurotium herbariorum* ser major
Mangin in Ann Sci Nat Bot

- (ser 9) 10 365 1909 4 glaucous group characterized by large ascospores. Would fall within 4 *echinulatus* q v 131
- 4 *Heterocephalus* Spring in Bull Acad Sci Belg 19 468 1902 This name was given to colonies in a bean egg which showed small heads globose and large heads columnar. Since no adequate figure or description was offered it may be designated as a nomen nudum
- Phyomyces heterosporus* Harz See 4 *atro ruber*
- P (*Micro-aspergillus*) *Hyckesii* figured by Biourge in La Cellule XXIII fasc 1 1 331 1923 (*Penicillium* Monograph) proved to be indistinguishable from 4 *gracilis* q v 139
- 4 *leptifolius* Sprengel in Sys Veg Ed III 4 541 1827 There is no suggestion of an *Aspergillus* in the description given
- 4 *holmensis* Biourge nomen nudum listed among the 4 *versicolor* group in Manuscript and in culture in his collection in 1939
- 4 *hortae* (Langeron) Dodge in Dodge Med Mycol p 628 1935 204
- Syn 8 *hortae* Langeron in Bul Soc Path Exotique 15 383-384 figs 1 3 1922 204
- Syn 4 *terreus* Thom 195
- 4 *humicola* Chaudhuri and Qachar in Ann Mycol 32 97 1934 Cited by Neill as 4 *versicolor* 193
- 4 *humus* Abbott in La St Coll J Sci 1 15-36 Fig 2a-c 1906 See also Louisiana Station Bul 194 One of the 4 *ustus* series 174
- 4 *hypophanthinus* Biourge was published as *Penicillium hypophanthinus* in Biourge Monogr La Cellule 33 fasc 1 pl 371 ? Pl XXII fig 130 1923 Afterward he transferred it to *Aspergillus* 4 *retractus* series 139
- 4 *incrassatus* Spring in Bull Acad Roy Belg 19 469 fig 2 1902 Not identical
- 4 *insigne* Winter (ex seccati in Rath Fungi No 173) in Hb Switz 1873 and Rath Krypt Hb Aufl 1 Abt II 2 61 1868 Initially not an *Aspergillus*
- 4 *insuetus* Buisson in Bull Soc Mycol France 24 85-87 Tab VIII figs 1 II 1918 4 *ustus* series 174
- 4 *intermedius* Speg in My Arg V in An Mus Soc Buenos Aires Ser 3 T 13 435 1911 In the 4 *niger* group
- 4 *islaconicus* Kinoshita in Botan Mag Tokyo 45 60-61 1931 Species diagnosis and figure reprinted in Acta Hystochim (Tokyo) 8(2) 21 1931 See 4 *glauca* group 14
- 4 *italica* Sacc in F Italici V 109 1881 Also in Michelis 1 91 1877
- Syn 4 *sterigmatophorus* Sacc in Atti d Soc Ven Tren d Sc Nat 2 fasc 2 231 Tabl XVII figs 5-8 1873 Some number of the 4 *candidus* group 11
- 4 *janus* n sp Raper and Thom in Mycologia 36 46-61 fig 1 1944 157
- var *brevis* Raper & Thom in Mycologia 36 561 63 fig 2 1944 170
- 4 *japonicus* Saito in Bot Mag Tokyo 20 61-63 1906 In the 4 *niger* group 231
- var *capillatus* Nakazawa Takeda and Suematu Culture available in Centralbureau 1939
- var or mut *grisea* Blochwitz in Ann Mycol 33 240 1935 It was a change of name only 4 *malitaceus* Mosseray
- 4 *javanicus* cited by Takahashi and Sakaguchi in Jour Agr Clem Soc Japan 1 No 10 1925 only in parenthesis after 4 *fumicola* s Wehmer and apparently deemed as synonym In 4 *niger* group

- A. jeanselmei* Ota in Ann de Parasitologie 1(2) 137-146 1923
A. flatus group 269
- A. leralitis* Ball in Amer Med 2 31 1901 This organism was found in an ulcer in the human cornea. Not identifiable 147
- A. longi* Oudemans in Arch Neerl Ser II V 7 281 Tab XIV 1902 Not since identified
- 1 *laneus* Link Obs p 16 1809
 Syn *Botrytis lanea* (Bonorden) Sacc in Syll 4 74
- 1 *laneus* in Schweinitz in Syn Am Bor is syn for *Rhinothricum curtissii* Berk in Grevillea 3 109 1875
- A. laokiaschanensis* Shih in Lingnan Sci Jour 15(3) 368 1933
 Near *A. unguis* in the *A. nidulans* group 169
- E. lateritium* Montagne in Century VI No 35 Ann Sci Nat Bot 3 Ser VI 154 1849 Sylloge II 257 One of the *A. glaucus* group with ascospores 7 to 10 μ in diameter
- A. Lepidophyton* Wehmer
 Syn *Epidermophyton concentricum* fide Dodge Med Myc 490 1935
- 1 *lignieresii* Cost and Lucet in Ann Sci Nat Bot 2 137 Pl 5 figs 19-23 1905 This culture from the lung of a penguin differs in cultural details from typical 1 *fumigatus* especially by the presence of swollen groups of cells in the mycelium 159
- A. longobasidia* Bainier nomen nudum a culture so labeled (Thom 4610 4771) was received from the Bainier Collection through da Fonseca. This was the type of 1 *bainieri* Mosseray in Ann Soc Sc Bruxelles 54 ser II p 77 1934 Member of the 1 *niger* group characterized by very long primary sterigmata 253
- 4 *lovaniensis* Biourge nomen nudum culture distributed by Biourge and appearing in NRRI Collection as No 76 See Thom and Raper U S D A Misc Publ 476 p 18 1941 117
- A. luckuensis* Inui in Jour Col Sci Imp Univ Tokyo 15 469 Pl 2 figs 1-8 1901 230
- var *rubeolus* Shih in Lingnan Sci Jour 15(3) 374 1933 230
- S. lutea* Bainier in Bul Soc Bot France 27 27 1880 *A. flatus* group See also Sartory and Jourde in Compt Rend Acad Sci (Paris) 146 548 549 1908 269
- A. luteus* (van Tieghem) Dodge in Dodge Med Myc p 675 1935
 Syn *S. lutea* van Tieghem in Bul Soc Bot France 24 103 1877
- A. luteo niger* (Lütz) Thom and Church in The Aspergilli p 160 1976 276
- Syn *S. luteo nigra* Lutz in Bul Soc Bot France 53 45 57 1907 Collected by A. Chevalier at San Thome Africa in fermenting seeds of *Theobroma cacao* In the *A. niger* group 276
- A. luteo niger* van Lwijk nomen nudum in Biourge's MS p 15 Probably attached to a culture of some black *Aspergillus*
- A. luteo virescens* Blochwitz in Ann Mycol 31(1/2) 73 83 1932 236
- In 1 *tamaris* series
- 1 *lutescens* Bainier nomen nudum described by Thom and Church in The Aspergilli p 193 1976 251
- In 1 *tamaris* series
- A. luticolor* Biourge nomen nudum listed in MS among the 1 *ochraceus* group in the Biourge Collection
- 1 *Macfie* Dodge in Dodge Med Mycol p 769 1935
- Syn *Sterigmatocystis* sp. Macfie in Ann Trop Med Parasitol 15 2 9 281 1921 Pathogen? In the 1 *niger* group 29

CHECK LIST OF SPECIES AND GENERA

347

- A. mac sporus* Bonorden in Handbuch d allg Myk 1851 fig 193 Not identifiable
- A. malignus* Lindt in Arch Exp Path Pharm 25 256-271 figs 111 1889 In *A. fumigatus* group 153
- A. maltracicus* Mosseray in La Cellule XLIII 265-266 Pl 4 figs 134 135 1931 A member of the *A. niger* group In the Biourge Collection 235
- A. mangini*
Syn *A. mino* (Mangin) Thom and Raper in U S D A Misc Publ 476 p 27 1941
Syn *F. herbariorum* var *minor* Mangin in Ann Sci Nat Bot (Ser 9) 10 365 1909
Member of the *A. glaucus* group with ascospores of intermediate size 127
- A. maximus* Lunk in Obo p 16 1899 Not an *Aspergillus*
- A. maydis* Quevedo in De Agronomica Nos 8 and 9 Buenos Aires 1912 See also Sartory in Champignons parasites de l'homme et des animaux p 532 1909 A species probably belonging to the *A. glaucus* group described in connection with disease in horses
- Emericella medias* Chowdhury and Mathur in Ann Mycol 36 61-63 1938 Probably *A. raris* color qv 147
- A. medius* Meissner in Bot Ztg 55 336-344 354-357 1897 See Thom and Raper U S D A Misc Publ 426 p 33 1941 163
- A. melleus* Yukawa in Jour Coll Agr Tokyo 1(3) 366 Taf XVII 1911 In *A. ochraceus* group 133
- A. menckii* Sartory et Flament in Compt Rend Soc Biol Paris 83 114 115 1920 See also Sartory et Bailly in Les Mycoses Pulmonaires et leur Parasites (Paris) pp 180-181 1923 1
- A. glaucus* group not since reported 146
- A. michelii* Irens in Linnaea 25 6 1852 Not recognized
- A. microcephalus* Moseray in La Cellule XLIII p 225-227 Pl 3 fig 42-49 1931 Member of the *A. nige* group In the Biourge Collection 233
- A. microsporus* Boker The description and figures given by Cattaneo and Oliva in Arch Lab Bot Critt Garovaglio 11 123 Pl 6 fig 9 1888 have been seen Not recognizable
- A. micro virido-citrinus* Constantin & Lucet in Ann Sci Nat Bot IX(2) 155 1905 In *A. flarus* group 263
- A. minimus* Wehmer in Bot Centralb 80 449-461 1899 See also Wehmer's Monogr p 79 1899-1901 Wehmer's culture was lost It has not been identified since
- A. minor* Baimier in Bul Soc Bot France 27 30 1880 Not recognizable Possibly *A. sydowii*
- A. mino* (Mangin) Thom and Raper in U S Dept Agr Misc Publ 476 p 27-29 1941 See *A. mangini* 127
- A. minutus* Abbott in Louisiana State Bul 194 1976 Also in Iowa St Coll Jour Sci 1 15-36 figs a b c and d 1976 See *A. uatus* group 14
- A. miyakoensis* Nakazawa Sino and Watanabe in Agr Chem Soc Japan Jour 12(9) 963-964 1936 See *A. nige* group 220
- A. mollis* Baimier and Sartory in Bul Soc Mycol France XXVII 453 Pl XVI 1911 Not *A. mollis* Berkeley See *A. glaucus* group 129
- A. mollis* Berkeley in English Flora V pt 2 p 340 1836 Not identifiable

- A. mongolicus* Biourge nomen nudum Biourge distributed under this name a culture essentially like *A. echinulatus* (q v) It appears in the NRRL Collection as No 137 See Thom and Raper U S D A Misc Publ 426 p 33 1941 132
- A. montenidensis* Tahce and Mac Kinnon in Soc Biol [Paris] Compt Rend 108 1007-1009 1931 See Thom and Raper U S D A Misc Publ 426 p 26 1941 In *A. glaucus* group 125
- A. mouthoni* Biourge nomen nudum listed by Biourge among his Les Furotrium in unpublished Manuscript Probably in culture in his collection
- A. mucoroides* Corda in Icones fungorum II 18 fig 76 1837 Probably some member of the *A. glaucus* group—unidentifiable
- A. mucoroides* Cook in Grevillea VII 9 1883 See *A. cookei* Sacc
- A. mulleri* Berkeley in Jour Linn Soc VIII 175 1873 Not identifiable
- A. mutabilis* Bainier et Sartory in Bul Soc Mycol France 27 458 pl VII 1911 In *A. glaucus* group 129
- A. mycetomi* Villabruzzii and Gelonci in Annali Med Nav Colon III 293 308 8 figs 1921
Syn *Madurella* sp undeterminable from the data available
- A. mycobanche* Link in Sp Plant Ed 4 6 69 1824 A fungus from rotting *Peziza*—not identifiable
- A. nantae* Pinoy in Compt Rend Soc Biol 97(19) 67-68 1907 This was probably *A. unguis* in the *A. nidulans* group Listed by Biourge as in his collection 170
- A. nanus* Montagne in Sylloge Generum Specierumque Crypt p 300 No 1112 Paris 1856 Also in Saccardo Sylloge Fungorum 4 p 71 Patavii 1886 In the *A. niger* group 231
- A. nanus* Oudemans, in Nederl Kruidk Arch Ser 3 2 1191 1904 Not *A. nanus* Mont q v Probably some conidial form in the *A. glaucus* group
- E. nebulosum* Fries in Sys Myc 3 334 1832 No data are given to link this with *Aspergillus*
- A. nicolletii* Pinoy cited by Biourge in 1939 manuscript He has apparently raised to species rank *A. nidulans* var *nicolletii* Pinoy q v 170
- A. nidulans* (Eidam) Wint in Rab Krypt Fl 12 62 1884 156
Syn *S. nidulans* Eidam in Cohn Beitr Biol Pflanz 3 397-411 Pl 20-22 1883
forme *Cesaris* Pinoy in Bul Soc Path Exot 8 11 1915 110
mut *coerulea* Blochwitz nomen nudum upon a culture in the Biourge Collection 1939 Listed in Biourge MS
mut *alba* F Wüll in Jour Bot (London) 1939 p 115 pl 618 150
var *lotus* Thom and Raper in Mycologia 31(6) 65 9 1939 159
var *nicolletii* Pinoy in Compt Rend Acad Sci Paris 144 396 1907 This variety was found fruiting within human tissue in a subject affected with Madura foot Listed in Biourge's Collection 1939 as *S. nicolletii* Pinoy 110
Syn *S. nidulans* var *nicolletii* Pinoy in Archives d Parasitologie 10 437-453 Pl VI 1906
- Diplostephanus nidulans* (Eidam) Langeron in Compt Rend Soc Biol Paris 87 343 315 1907

- Syn for *l. nilans* Fidan
q v
- A. niger* van Tieghem in Ann Sci Nat Bot Ser 5 1 8(4) 210 186 See also Thom and Cur ne in Jour Agr Res 7 1 15 1916
- Syn *S. niger* van Tieghem in Bul Soc Bot France 24 10 103 1877
- Syn *Aspergillus niger* (v Tieghem) Speg in Myc Arg 1 in Ann Mus Vac Buen s Aires (ser 3) 13 435 1911
- mut *cinnamomeus* in comb See *A. cinnamomeus* Schiemann 223
- var *laevis* Blochwitz in Ann Mycol 33 219 1935 Proposed for smooth spored strains *niger* var *sementarius* Nakazana Simo and Watanabe in Jour Agr Chem Soc Japan 144 171 2 and 184 1936 (in Japanese) 220
- mut *suave* Blochwitz in Ann Mycol 32 8 1934 In the *A. niger* group Such a separation based upon conical color is of doubtful valid ty
- mut *Schiemannii* in comb See *A. schiemanii* (Schiemann) Thom 224
- forma Tuebingen Schober in the Centraalbureau is apparently the strain used in the researches of Schober
- A. niger citricus* Wehmer name on a culture received from Neuberg (C T No 4668 4) but without description
- Syn *l. citricus* Mosseray q v
- S. nigra* Bainier Syn for *S. phoenicea* (Corda) Fatouillard and Delacroix in the *l. niger* s ries
- A. n. grisea* Robin in Histoire Naturelle des Vegetaux Paris (Paris) p 518 pl 5 fig 2 1853 No succ ss has been made in interpreting Robin s organism 151
- A. nigricans* Wreden in Compt Rend Acad Sci (Paris) 368 1867 Probably *l. niger* group
- l. nigriceps* B and C in the Curtis Collection Specimen collected by Charles Wright (No 977) in Cuba Cited by Cooke V C in Crevillia 17 21 Sept 1858 A slide from this material prepared by Bullard and preserved in the Harvard collection shows a characteristic organism of the *A. niger* series not separable from *l. niger* van Tieghem
- l. niceocandidus* Fildau in Rabh Krypt Fl 8 151 190 In the *l. candidus* group
- l. nireo glaucus* Thom and Raper in U S Dept Agr Misc Pub 496 p 35-36 1911 130
- A. nireus* Blochwitz in Ann Mycol 27(3/4) 205-206 Taf III fig 2 1929 A member of the *l. reus* group
- var *majo* Blochwitz in Ann Mycol 32(1/2) 86 1934 4p Apparently belongs in the *l. candidus* group
- var *nubila* Blochwitz in Ann Mycol 32(1/2) 83 1934 See *A. coelestis* 211
- A. voltig* Hallier in Zetschr Parasit (Not found) cited by Cattaneo and Oliva in Arch Lab Bot Critt Garovaglio 5 192 1883 Not recognizable to Wehmer culture in the Centraalbureau at Baarn was identified by Thom and Raper as *l. pseudo glaucus* 111
- E. oblitum* Schw Syn fungorum in Amer Bor 27 5 Some unidentifiable member of *l. glaucus* group
- A. oblongus* E and C No 760 in Nuttall s Flora of Fayette County West Virginia Listed apparently by Millsbaugh in The Living Flora of West Virginia in W Va Geol Survey

- 5(A) 32 1913 as 1 *glaucus*
var *oblongisporus* F & W
Examination of this material
shows it to be a mixture of 1
flavus and *A. repens*
- S. ochracea* Brunier in Bul Soc
Bot France 22 78 1881
Some member of the 1 *ochraceus*
group
- S. ochracea* Delacroix in Bul Soc
Mycol France 7 109 Pl VII
fig f 1891 252
- Syn *S. delacroixii* Sacc in Syl
loge 10 57 Delacroix de
scribed his organism without
recognizing the previous use
of the specific name 252
- S. ochracea* (Wilhelm) Schroter
See *A. ochraceus* Wilhelm
- A. ochracea ruber* Sacc in Michx
lia I 77 1877 (Saccardo P
A No 1063 in Myco Veneta on
bark of Walnut 1816 see fig 17
in Fungitalia) Some conidial
form of the *A. glaucus* group
- A. ochraceus* Wilhelm Inaug Dis
Strassburg p 66 1877 270
- var *microspora* Tiraboschi in
Annali di Botanica 7 14
1908 281
- S. ochroleuca* Speg in Anal Mus
Nac Buenos Aires Ser 3 t
13 431 1911 In the 1 *ochra*
ceus group 276
- 1 *ochroleucus* Haller in Enum
Method Stirp Helvet Indig t
I p 6 174 Hist Stirp t
III 1768 Cited by (aspergillus
as questionable syn for 1
elegans
- 1 *ola akii* Okazaki in Centralt f
Bakt 2 abt 42(10 14) 225
1914 1 *candidus* group 211
- This is cited in the Sylloge 22
1760 as *S. ola akii* (Saito) Sac
cardo
- A. oligosporus* Corda in Icones III
Tab II Not an *Aspergillus*
- S. olivacea* van Tieghem in Bull
Soc Bot France 2 103 1855
Not identified with *A. olivacea*
- Preuss 1852 Not identifiable
- 1 *olivaceo fuscus* Mo seray in La
Cellule XLIII 258 259 pl 3
fig 49-50 1931 A member of
the *A. niger* group in the
Biourge Collection 231
- Cladosporium olivaceum* Vuill and
Vuill in Trans Brit Myc Soc
22 191 200 Pl 11-13 1938 73
- 1 *olivaceus* Delacroix in Bul Soc
Mycol France 13 118 170 text
fig 1897
- Syn 1 *delacroixii* Sacc and
Sydon q v Not *A. olivaceus*
Ireu s 1857 Probably in 1
glaucus group
- A. olivaceus* Preuss in Linnaea 25
77 1852 Not identifiable
- 1 *Omiki* on a culture from Okunuki
in the Centraalbureau collection
No description found Bloch
with cites it in Ann Mycol 22
240 1935 As 1 *ochraceus*
- A. odosporus* Wallroth as Flora
Cryptogamica Germaniae No
1928 in Compendium Florae
Germanicae 4 296 1833
Some one of the 1 *glaucus*
group
- 1 *oriolus nomen nudum* attributed
to Biourge Distributed by
Biourge Collection and appears
as NRRI No 87 It was iden
tified as a strain of *A. chevalieri*
by Thom and Raper in U S D
A Misc Publ 496 pl 21 1941 170
- 1 *oryae* (Ahlburg) Cohn in
Jahresb Schles (en für Vaterl
Cultur 21 276 1863 In the
1 *flavus-oryae* group 271
- Syn *F. oryae* (Will.) Korschelt
Name with incomplete descrip
tion of same organism published
in Dinglers Polytech Jour
230 330 1855 251
- 1 *oryae* var *basidiferens* Constan
tinian & Lucet in Ann Sci Nat
Bot IX (2) 16 1905 In
the 1 *flavus-oryae* group
- Syn 1 *oryae* var *basidifer* Sacc
Syn 2

- A. ostianus* Wehmer in Bot Centralb 80 413-461 1899 Monogr pp 11 119 Taf II No 1 1899-1901 In the 1 ochroceus group 283
- A. oralispermis* Link in Ols II p 37 1816 also in Sp Plant Id IV Vol VI(1) 60 1821 Cite Las synonym of *A. odoporus* Wallr (1833) without evidence of identity or reason for redescription
- 1 *panamensis* Haper and Thom in Mycologia 35 565 572 fig 5 1944 217
- A. pauciculus* Speare in Hawaiian Sugar Planters Exp Sta Path & Physiol Ser Bul 12 p 38 11 3 and 4 1912 In the 1 flarus group 266
- Syn *A. flarus* var *viridis* Bloch witz q v 267
- 1 *penicillatus* Greville in Scottish Cryptogamic Flora 2 37 plate 3 1923
- Syn *A. glaucus* (conidial) not identifiable to species
- A. penicillatus* Link in Sp Plant Id 4 6(1) 69 1821 The description given by Link is not sufficient to identify any form but since he probably intended to cover the organism of Greville it may be assumed to be conidial *A. glaucus* Sturmeste it as *Drieda elegans* without specifying its reasons
- A. penicilloides* Spegazzini in Rev Agrar Veter La Plata p 245 1896 In the 1 restrictus series 14
- A. penicillopsis* (Hennings) Racib I Hennings as *Stilbothamnium penicillopsis* I Henn & F Nym described in Fungi Mon subensis (Warburg Monsumia Bd I p 37 (Leipzig) 1899 Exsiccati of type in Pathological collections U S Dept Agr Bur of Plant Industry as Raciborski No 87 in Crypt Paras Java
- Seal of Laras Alig uleiz Javas II 7 1900 287
- 1 *periconostoides* Sacc in Ann Mycol 11 370 1913 No 19a in Sydow Fungi exotici exsiccati collected by P W Craff on leaves of Carica in Luzon 1912 Not identifiable
- 1 *perniciosa* Inui in Jour Coll Sci Imp Univ Tokyo 18 473 1901 Inui recorded a yellowish greenish color in the mycelium of this species which is regarded as related to *A. lachnensis* and *A. niger* 230
- 1 *pertusa* is a nomen nolum on a culture distributed by Biourge The organism was close to *A. restrictus* series 147
- 1 *petiolatus* Haller in Historia stirpium indigenarum Helvetiae innotata etc 1 68
- 1 *phacocephalus* Durieu and Montagne in II Alg p 31 1849
- Syn *A. phacocephala* (Dur et Mont) Saccardo Fungi italici fig 903 and No 1244 in Sacc Myc Veneta 1877 One of the 1 niger group
- 1 *pharicis* (Corla) Tien in The 4 pergilla p 12 1920 272
- Syn *A. pharicis* (Corla) Patwill and Delaer in Bul Soc Mycol France 7 111 Pl 9 1891 273
- Syn *Ustilago phoenicis* Corda in Icones Fungorum 4 9 pl 3 fig 96 1840 One of the 4 niger group 273
- A. picta* Blanchard cited by Castellani & Chalmers in Man Trop Med p 806 1913
- Syn *Trichophyton picta* Blanchard in Traite Path Gen II 919 1906 The production of polytelomate cultures suggests relationship to *A. versicolor*
- A. pollinis* Howard in Am Bee Jour 36 517 58 1936 See also id m 50-531 1899 This was *A. flarus* fide Turesson

- in Svensk Bot Tidskr 11 30 1917 266
- S polychroma* Ferraris in Fl It Crypt Hyph p 640 1906 193
- Syn *A versicolor* q v 190
- A polychromus* De Mello in Jour Indian Bot 1(5) 158-161 1920 The data given are not sufficient to distinguish whether he had 1 *nidulans* or *A sydowii*
- A polychromus* Sartory Sartory and Meyer Cit Syn of *A versicolor* fide Blochwitz in Ann Mycol 31 73 1933
- A polymorphus* Moquin Tandon in Elements Bot Med 2 Ed 469 1866 Not identifiable
- A pouchetii* Montagne in Ann Sci Nat Bot 4 Ser 6(12) 182-183 1859 As described this was one of the mucors noted as having resemblances to *A maximus* (Sporodinia)
- A praecox* Mosseray in La Cellule \LIII 229 1934 Cited as synonym of *A fuliginosus* Peck 233
- S prasina* Baimier in Bull Soc Bot France 27 31 1880 Not identifiable
- A profusus* Hann nomen nudum Cited by Thom and Raper in discussing *A scheelei* Baimier and Sartory (Bul Soc Myc France 28 257 1912) as nearly related to *A scheelei* See Thom and Raper U S D A Misc Publ 476 p 13 1941 Syn of 1 *pseudoglaucus* 111
- A proliferans* Geo Smith in Brit Mycol Soc Trans 26(1/2) 26 Pl III 1943 In the *A ruber* section of the *A glaucus* group 117
- 1 *pseudo carbonarius* (Baimier) Mosseray in La Cellule \LIII 2:1 225 Pl 3 fig 7-13 1934 233
- Syn *S pseudo carbonaria* nomen nudum on culture (Thom 4680 482) from the Baimier collection
- 1 *pseudo citricus* Mos. eray in La Cellule \LIII 275-279 pl 4 figs 103-104 1934 Member of the *A niger* group in the Biourge Collection 233
- A pseudo-claratus* Putjewicz in Schrift Naturforsch Gesell haev 16 2 p 309 1900 See Saccardo Sylloge Fung 16 p 1078 In *A claratus* group 98
- A pseudo-elatior* Mos. eray in La Cellule \LIII 255-256 pl 3 figs 33-37 1937 Member of the *A niger* group in the Biourge Collection 1939 234
- A pseudoflavus* Saito in Centralbl f Bakt 2 abt 18(1/3) 31 figs 15-18 1907 966
- Syn *S pseudoflava* Sacc Sylloge Fungorum 22 1260-1260 The morphology given indicates that 1 *pseudoflavus* is one of the intermediate forms which bridge the gap between typical 4 *flavus* and *A oryzae*
- A pseudoglaucus* Blochwitz in Ann Mycol 27 207 1929 Emended description by Thom and Raper in U S D A Misc Publ 476 p 12 1941 *A glaucus* group 110
- S pseudo nidulans* Vuillemin Arch Parasitologie 8 540-541 1904 Vuillemin transfers the ascosporic form described by Grijns as 4 *fumigatus* in Centralbl Bakt II 11 330 1903 to this specific name emending Grijns's description by indicating the double nature of the band by which he separates his form from *A nidulans* as described by Eidam This discussion by Vuillemin tallies with the commonest of our American soil forms of 4 *nidulans* but not with the description by Grijns
- 1 *pseudo niger* Mosseray in La Cellule \LIII 256-258 Pl 4 figs 113-117 1934 A member of the 1 *niger* group in the Biourge Collection 1939 231

- S. pseudo nigra* Constantin and Lucet in Bul Soc Mycol France 19 33-41 1903 In the *A. niger* group
- A. pseudo Schiemanis* Biourge nomen nudum cited from Centralbureau catalogue 1931 as an actively diastatic organism represented in the Biourge Collection
- A. pulchellus* (Speg.) Thom and Church in The Aspergilli p 181 1906 229
Syn *Aspergillopsis pulchella* Speg in Myc Arg V An Mus Nat Buenos Aires Ser 3 T 12 436 1911 In the *A. niger* group 229
- E. pulcherrimum* Winter in Rabh Krypt Fl 2 auf 1 Abt 2 60 1887 Noted there as also in Herbarium of Winter and in Hansen Fungi fimeoli Danici 1041 of Sep Abdr *A. coprophilus* form from the dung of foxes in Leipzig and dogs in Denmark by Hansen not an *Aspergillus*
- A. pulmonum hominis* Welcker Discussed by von Dusch in Virchow's Archiv (N F 1) 11 561 566 1857 Apparently *A. fumigatus* 151
- A. pulverulentus* (McAlpine) Thom in Jour Agr Res 7 10-11 1916 223
Syn *S. pulverulenta* McAlpine in Agr Gaz N S Wales 7 307 1896 In the *A. niger* group 223
- A. pulvinatus* B and C original collection as far as seen appears to be in the Curtis Collection from Society Hill S C 1855 also one marked F cub —Wright No 642 in the Curtis Collection another series of specimens of B and C No 1643 in Ellis and Everhart V *A. Fungi* collected on dead twigs at Newfield N J 1885 Also No 2306 in the Ellis Collection Not an *Aspergillus*
- S. purpurea* van Tieghem in Bul Soc Bot France 24 101 103 1877 Possibly *A. nidulans*
- A. purpureofuscus* Fries in Sys Myc 3 388 1879 Probably the same as *A. purpureofuscus* of Schweinitz
- A. purpureofuscus* Schweinitz in Synopsis fungorum in America boreali media degentium Secunsum observationes In Trans Amer Phil Soc N S 4 287 No 7680 1831 Also in Saccardo Sylloge Fungorum 4 68 Patavi 1886 Not an *Aspergillus*
- A. purpureus* Haller Historia stirpium indigenarum Helvetiae inchoata etc 1 68 Not recognisable
- S. pusilla* Peyronel in I genu atmospherici dei funghi con micelio Thesis Padova p 91 1914 See the *A. nireus* series 203
- A. pusillus* Massee in Kew Bull Misc Inf 4 154 1914 From Soil Sudan Not identifiable
- A. pyri* English nomen nudum name published in Research Studies of the State College of Washington VIII (3) 127 1940 (Doctoral Thesis Taxonomic and pathogenicity studies of the fungi which cause decay of pears in Washington) Subsequent work by English led to recognition of the name as a synonym of *A. niger* 231
- A. quadrifidus* Link in Obs 2 36 1816 Probably not an *Aspergillus*
- A. quadrilineatus* Thom and Raper in Mycologia 31(8) 660 fig 3D and 4B 1939 160
- A. quercinus* (Bainier) Thom and Church in The Aspergilli p 186-187 1906 276
Syn *S. quercina* Bainier in Bul Soc Bot France 23 78 1881 776

- 1 *quininae* Heim in Bul Soc Myc France 9 239 1891 The culture was found upon quinine solution but the description given will not separate it from *A fumigatus*
- A racemosus* Persoon in Neues Mag Bot 1 121 1794 Also in Tentamen Disp Meth Fung p 41 1797 Not recognizable
- A ramosus* Halber in Ztschr f Parasit 2 266-269 Pl 6 figs 1-6 1870 The figures and descriptions evidently represent a strain of *A fumigatus* 151
- A raulini* nomen nudum in Biourges table probably attached to a culture in his collection
- 1 *rehmii* Zukal in Oesterr Bot Zeitschr 43 160 Pl II figs 1-10 1893 Zukal regarded this form as close to *S sulphurea* Fresenius but his description of perithecia and ascospores excludes the *A ochraceus* group Blochwitz evidently believed that Zukal had a mixed culture hence the name would be untenable Cultures belonging to the *A ochraceus* group have been distributed under the name but without proving their authority 251
- A repandus* Rainer and Sartory in Bul Soc Myc France 27 463 Pl XVIII 1911 *A glaucus* group 133
- 1 *repens* (Cda) Delbary in Abhandl I Senkenberg Naturf Gesell ch 7 39 1870 103
- A repens* Delbary and Woronin in Beitrage zur Morphologie und Physiologie der Pilze p 379 1866
- Syn *A glaucus* var *repens* Coria in Icones 5 53 Taf II fig 21 1817 103
- E repens* var *amstelodami* Vuill Soc Mycol de France Bul Trim st 35 131 1970 172
- A restrictus* G Smith in Jour Text Inst 22 T 115 fig 5 1931 141
- A restrictus* var H C Smith in Jour Text Inst 22 T 115 figs 4 III and E 1931 See *A restrictus* series in *A glaucus* group 141
- A roseus* Batsch in Elenchus Fungorum p 183 No 58 fig III 1783 Cited by Link Spec Pl ed IV t VI pt 1 p 68 1874 Also by various authors for a rosy or flesh colored organism and by Corda as *Haplotrichum roseum* in Pracht flora Pl VI
- 1 *roseus* Link in Sp Plant ed IV t 6 part 1 p 68 1821 Link took the name *roseus* used descriptively but not nomen clatorially by Batsch (Elenchus Fung p 183 no 58 fig 58 1783) for a mold presumed to have been an *Aspergillus* by later authors The name appears III No 2774 in the Curtis Collection (1849) for a member of the *A candidus* group Neither descriptions nor specimen dating back to these authors fix this name for any definite series
- 1 *rubens* Creen in Boston Soc of Med Sc 1868 Not identifiable
- 1 *ruber* (Bremer) 114
- Syn *A ruter* (Speckermann and Bremer) Thom and Church in The Aspergilli 112 1976 114
- Syn *Eurotium rubrum* Bremer in Zeitschr f Untersuch d Nahrung und Genussmittel IV 1901 p 72 also in Die fettverzehr Organismen in Nahr u Futtermitteln Disert Munster 1907 114
- Syn *F rubrum* Speckermann and Bremer in Landw Jahrb 31 81-124 1907 114
- 1 *ruber* Estienne
- Syn *Phycomyces heterosporus* Harz

- Syn S rubra* (Latiene) Bourge
Syn Monascus purpureus cer
 tainly not an *Aspergillus*
S. rubescens nomen nudum in a cul
 ture in the Hunter Collection
 (Thom No 4640-48) 1
faripis group
A. rufescens Berlese in Fungi Mori 191
 color Fasc VII No 4 Tav 51
 figs 17-18 1889 1x bulb
 conical strain of *glauca*
 group
rugulosus Thom and Raper in
 Mycologia 31(8) 661 n fig 37
 and 4C 1939
A. rutidans Moray in La Cellule
 VIII 231 233 Pl 4 fig 69-
 90 1934 A member of the
 niger group in the Bourge
 Collection 1939
A. sachari Sprezzani in Anales del
 Museo Nacional de Buenos
 Aires 6 Ser 2 3 244 1909
 Some insufficiently described
 member of the *glauca*
 group
A. sachari Chauflour and Schar in
 Ann Mycol 32 83 1934
 Blochwitz in Ann Mycol 23
 1910 1933 leaves this species in
A. quercinus
A. salmonae Bourge nomen
 nudum cited by Henrard in La
 Cellule VIII fasc 7 p 33
 1934 as one of the series
glauca. On p 30 *demise*
 records that single ascospores re
 quired 3 months for germination
 and that this species was found
 to be homothallic. Identifica
 tion to species within the *A.*
glauca group is not possible
 without more information
Allospora Sapucaya Pim in Proc R
 Acad 1883 and in Jour Bot
 1883 p 234 One of the
 niger group causing rot in allia
 ceous bulbs
sa to yz nomen nudum was
 attached by Bourge to a culture
 of an organism close to *f*
gracilis in the *restrictus*
 series
sa to yz Sydow in Ann Mycol 139
 11 186-160 Pl VIII 1913
 10 111 in *l. lama* group
f. scheelei Hunter and Sartory in
 Bul Soc Myc France 23 24-
 7 11 X 1917 See Thom
 and Raper in *l. c. D. A. Muc*
 Full No 476 *l. p.* 1911 4
glauca group
f. scheelei Hunter and Sartory var
ff. ste. See *l. epens* series
 in *l. glauca* group
f. schiemanii (Schiemann) Thom in
 Jour Agr Res 7 13 1918
 See also *l. fuscus* Schieman
 name changed because previ
 ously used
S. schneegiana Bourge nomen
 nudum in Bourge's Myc listed
 in the *l. candidus* group as
 applying in a culture received
 from Selmege
f. scleritise Moray in La Cellule
 VIII (fasc 7) 24 244 1934
l. member of the f. niger
 group
A. sclerotium Huber in Phyto
 pathology 23(3) 306-8 fig 1
 1933 *l. heavy sclerotium*
 producing member of the *l.*
ochraceus group
f. conjunctus Raimier et Sartory
 in Bul Soc Myc France 27
 340-363 Pls V VI 1911 An
 ascospore form of the *l. glauca*
 group
f. semisimile Marchal in C R
 Soc R B Belg 33 193 Pl
 II fig 3 1934 Not an *Asper*
gillus
A. siebenmannii Constantin and Lucet
 in Ann Ser Nat Bot 9 2 p
 167 1905 This name is based
 upon Siebenmann's description
 of an organism from the human
 ear identified by Siebenmann as
f. flavus but regarded by the
 describers as a separate species

- based upon the description given by Siebenmann 266
- 4 *simplex* Persoon in Tent Disp Meth Fung p 41 1797 Tradition calls it a *Penicillium*
- 4 *soya* on a culture from Okunuki in the Centraalbureau collection No description found cited by Blochwitz in Ann Mycol 33 240 1935 as *f. flavus*
- 5 *skottsbergii* Bresidola and Vester gren no 200 in Vester gren Micromycetes rariores selecti Rossia baltica ins Oslia Kiel kond in silva abiegna prope Kattiel in folis vivis Aquilegiae vulgaris 1899 Distributed without description examined in collaboration with Professor Thaxter at the Harvard University Cryptogamic Herbarium did not prove to be an *Aspergillus*
- 1 *spadix* Amons in Arch v Suiker industrie in Nederlandsch Indie 29 12 14 1921 This is one of the *f. tamaris* series 207
- 4 *sparvus* Raper and Thom in Mycologia 36 572-574 fig 11 1944 283
- 1 *sphaerospermus* Corda in Icones II 18 1854 No description
- 4 *spiralis* Grove in Journal of Bot 23 164 tab 257 fig 5 1880 A conidial organism of the *f. glaucus* group
- 1 *spirius* cited by Amons in Arch v d Suikerindustrie in Nederlandsch Indie 29 14 1921 Not identifiable
- 5 *spuria* Schroeter in Cohn Kryptogamen Flora von Schlesien 3 2 Hälfte Iief 1 p 218 1893 301
- Syn *S. carnea* van Tieghem 1877 See *A. carneus* (van Tieghem) Blochwitz 301
- 1 *stellatus* Cursi in Rend Acad Naz Lincei II 421-426 fig 1 1934 Culture in Centraal bureau list 1937
- Syn *f. varicolor* 163
- F. stercoraria* Han en in Meddelelser fra den Naturhist Foren i Kjobenhavn p 310 1876
- Syn *Anaxiopsis stercoraria* Hansen in Bot Ztg 33 127 131 Tab II fig 8 1897
- 1 *stercoreus* Sacc in Michelia 1 75 1877 Also Fungi italici no 19 An unidentifiable member of the *A. glaucus* group
- 1 *sterigmatophorus* Saccardo in Atti Soc Ven Tren Sci Nat 2 fasc 2 232 Tab VII fig 5-8 1873 211
- Syn *S. italica* q v 211
- 1 *strychni* Lindau in Hedwigia Bd 43 Heft 6 306-307 1904 273
- Syn *A. pulverulentus* McAlpine 1896 In the *A. niger* group 223
- 4 *subfuscus* Johan Ol en in Meddelelser fra Naturh forening i Kristiania 1880 Cited also in O Johan Olsen Videnskapselskabet i Kristiania p 21 1886 Some member of the *f. niger* group 231
- 4 *subgriseus* Peck in Bul Torrey Bot Club 22 5 210 1895
- Syn *F. subgriseum* Peck in Rept N Y State Mus Bot p 30 1910 Also in N Y State Mus Bul 140 1911 There is no way to identify Peck's species
- 1 *sulphureus* (Des) Thom and Church in The Aspergilli p 185-186 1926 270
- Syn *S. sulphurea* Fre enius in Beitr / Mykologie Heft 3 p 83 Tab VI fig 30-33 1863 Al Silloge 4 73 1866 In *f. ochraceus* group 25
- 1 *sydowii* (Bainier and Sartory) Thom and Church in The Aspergilli p 147 148 1926 184
- Syn *S. sydowii* Bainier et Sartory in Ann Mycol 11 25-29 11 III 1903 *f. sydowii* series 184
- var *achlamyosporus* Nakazawa Sim and Watanabe in Jour Agr Chem Soc Japan 10(2) 18-19 1934 The absence of

CHECK LIST OF SPECIES AND GENERA

357

- Holle cells is inadequate for separation
- A. syncephala* Curguen in Champ 186
Parasit Hom Anim p 16; fig
6 1901 Probably *A. fumigatus*
- S. servatiana* Moers in Bot Közlem 151
19 44-66 13 figs 1971 One of the *A. candidus* group
- A. tabacinus* Nakazawa Sino and 186
Watanabe in Jour Agr Chem Bakt I 35 140 1903 Cited by
Soc Japan 10(2) 177 193 deaux 32 317 1907 Wehmer's
1934 appears to have been a culture was one of the *A. glaucus*
strain of the *A. versicolor* group but not the pathogenic
series organism causing the disease
1 *tamaris* Hita in Centralb f 193
Bakt etc 2 Abt 3 No 14/16
pp 432-457 1913 *A. tamaris*
series
- A. tardior* Bourge nomen nudum 254
listed among his cultures of the
A. restitutus group (Bourge's
See 1 *Microaspergillus*)
- A. terreus* Thom in Turesson Cote 18
Svensk Botanisk Tidskrift 10
5 1916 Without description
diagnosis Thom and Church
Amer Jour Bot 5 85-86 1918
The *A. terreus* series
- var *aureus* n var 195
var *Boedijnii* (Bloch) n comb 193
Thom and Raper
- Syn *A. Boedijnii* Blochwitz Ann 197
Mycol 32(1/2) 83 1934
- var *floccosus* Shih in Lingnan 198
Science Jour 11 377 pl 16 fig
3 1936
- var *subfloccosus* Shih in Lingnan 198
Science Jour 18 371 pl 16 fig
4 1936
- A. terricola* Marchal in Rev Mycol 15(89) 101-103 1933 In *A. tamaris* series -53
- var *Americana* Marchal in Thom 125
and Church Am Jour Bot 8 1919 Also in Thom and
Church The Aspergilli p 197 1926
- A. thomii* This was an undescribed 253
sclerotium producing strain of
A. flavus sent by P W Graff to
- Amer 186
tion Type Cult Collec
A. tiraboschi Carbone in Atti d 766
Inst Bot Univ Iavia Ser II
Vol XI 379 1917 In the
1 *sydowii* *versicolor* group 186
1 *tololau* Wehmer in Centralb
Bakt I 35 140 1903 Cited by
Dubreuil in Jour Med Bor
deaux 32 317 1907 Wehmer's
culture was one of the *A. glaucus*
group but not the pathogenic
organism causing the disease
1 *tololau* Dodge Med Myc 490
1935 calla it Epider
mophyton
- S. tropicalis* Matta in Bol Inst 18
Brasil Sci 3 51 54 2 figs
197 Probably *A. sydowii* but
not separable from other strains
1 *tubingensis* (Schleber) M. seray in
La Cellule XIII 715-21 pl
3 fig 58-60 1934 Member
of the 4 nig group in the
Bourge Collection 1937
- 1 *tunetanus* (Langeron) Dodge in 18
Dodge Med Mycol p 635
1935
- Syn *S. tunetana* Langeron in Bul 18
Soc Isth Prot VII 315-34
illustr 1921 See *A. sydowii*
series
- 1 *umbriae* Patterson in Bull 18
Torrey Bot Club 25 234 1900
In the *A. tamaris* series
- 1 *umbrosa* Baimier and Sartory in 169
Bul Soc Mycol France 28 767
Pl XII 191 In the *A. glaucus* group
- 1 *unguis* (Emile Weil and Gauhin) 179
Frend Thom and Raper in
Mycologia 31(6) 661 8 fig 6
1939
- Syn *S. unguis* Emile Weil and 169
Gaudin in Arch Med Exp
Anat Isth (Paris) 28 463-469
1919 See Thom and Raper
The 4 nidulans group Mycolo
gia 31 667 1939
- A. ustus* (Baimier) Thom and Church 169
in The Aspergilli p 157 153
1926

- Syn *S. usta* Buhner in Bul Soc Bot France III 78 1881 *A. ustus* group 171
- var *laevis* Blochwitz in Ann Mycol 32(1/2) 84 1934 175
- A. ustilago* Beck in Itin Prin S Coburgi 2 148 (Wien) 1888 Saccardo in Sylloge Fungorum III 5% (Patavi) 1897 223
- Syn *A. phoenicia* in the *A. niger* group 222
- A. lancampenhouti* Mattlet in Ann Soc Belge Med Trop 4 161-176 1971 Name only *ibid* 8 31 1926 In the *A. versicolor* group 193
- S. varia* Baimier in Bul Soc Bot France 27 30 1880 Probably some nonasco poric *A. nidulans* or *A. unguis*
- A. variabilis* Gasperini in Atti Soc Toscana Nat Sci Pisa Mem 8 fasc 2 p 376 1887 From the description this was probably some strain of the *A. flavus* group 266
- A. varians* Ceni in Rivista sperimentale di Freniatria p 31 1905 Certainly identified by Tiraboschi in Annali di Bot 7 9-10 1908 as *A. versicolor*
- A. varians* Wehmer in Bot Centralb 80 460-1 1899 Also in Wehmer Monogr pp 77-79 taf 1 1899-1901 If Wehmer's description is correct his species is not known in culture now. The authors have believed that *A. varians* is identical with *A. itaconicus* 142
- A. varicolor* (Berk. and Br.) Thom and Raper in Mycologia III 663 667 fig 4D and fig 5 1939 163
- Syn *Fmericella varicolor* Berk. and Br. in Introd Crypt Bot p 340-341 fig 16 185 See Patouillard in Bull Soc Myc Fr 7 43-49 pl 4 fig 6-12 1891 163
- Syn *In engea eryth. aspora* Borsini Jahrb Wiss Bot (Erlangenheim) 16 450-463 pl 19 20 (1884) 1885 163
- Syn *Fmericella medias* Chowdhury & Mathur Ann Myc 36 61-63 1938 163
- Syn *A. stellatus* Curzi Rend Acad Naz Lincei 19 p 424-425 fig 1 1934 163
- A. variegatus* Mosseray in La Cellule XLIII 238-239 pl 4 fig 72-75 1934 Member of the *A. niger* group, in the Biourge Collection 1939 233
- A. velutinus* Mosseray in La Cellule XLIII 252 253 pl 3 fig 38-41 1934 Member of the *A. niger* group in the Biourge Collection 1939 234
- S. veneta* Massalongo in Bul Soc Bot Ital Vo 7-8 p 159 1900 Not cultivated and not identifiable by description although Werkenthin (Phytopathology 6 247-249 1916) used *A. venetus* for strains now known to be *A. terreus*
- E. verruculosum* Vuillemin in Bul Soc Myc France 34 83 1918 See *A. echinulatus* in *A. glaucus* group 131
- A. versicolor* (Vuillemin) Tiraboschi in Ann Bot (Rome) 7 9 1908 190
- Syn *S. versicolor* Vuillemin cited by Minsky in Thèse de Med Nancy No 27 p 16 1903 *A. versicolor* series 190
- mut *coerulea* Blochwitz in Ann Mycol 27(3/4) 201 1929 Represents a pure blue strain arising from a green strain. Probably *A. aglowi*
- var *fulvus* Nakazawa Takeda and Saematsu culture available from the Centralbureau 1939
- var *glaucus* Blochwitz in Ann Mycol 32 86 1931 A strain greener than typical for the species from human skin accompanying a Trichophyton. Similar green strains have been observed by us 197

CHAPTER XXV

ACCEPTED SPECIES, VARIETIES, AND MUTATIONS

<i>A. alliaceus</i> Thom and Church	244	<i>A. janus</i> var <i>brevis</i> Raper and Thom	190
<i>A. amstelodami</i> (Mang.) Thom and Raper	122	<i>A. japonicus</i> Saito	231
<i>A. atropurpureus</i> Zimmerman	226	<i>A. luchuensis</i> Inui	230
<i>A. atenaceus</i> G. Smith	246	<i>A. lutescens</i> (Bain.) Thom & Church	251
<i>A. auramori</i> Nakazawa	270	1 <i>mangini</i> n. comb.	174
<i>A. butyracea</i> Bainier	282	<i>A. medius</i> Weissner	133
<i>A. caespitosus</i> Raper and Thom	166	1 <i>melleus</i> Yukawa	279
<i>A. candidus</i> Link	207	<i>A. micro virido citrinus</i> Cost and Iucet	263
<i>A. carbonarius</i> (Bain.) Thom	229	<i>A. miyakoensis</i> Nak. Simo & Wat.	220
<i>A. carneus</i> (v. Tiegh.) Bloch emend.	20	<i>A. monticidensis</i> Talice and Mac Kinnon	125
<i>A. carnosus</i> (Bourge) Thom and Raper	134	<i>A. nidulans</i> (Eidam) Wint.	156
<i>A. chevalieri</i> (Mang.) Thom and Church	118	<i>A. nidulans</i> (Eidam) Wint. mut. <i>alba</i> Yull	159
<i>A. chevalieri</i> (Mang.) Thom and Church var. <i>intermedius</i> Thom and Raper	121	<i>A. nidulans</i> (Eidam) Wint. var. <i>latus</i> Thom and Raper	159
<i>A. citrisporus</i> von Hohnel	251	<i>A. niger</i> van Tieghem	216
<i>A. clavatus</i> Desm.	97	<i>A. niger</i> v. Tiegh. mut. <i>cinnamomeus</i> (Schuem.) n. comb.	223
<i>A. conicus</i> Blochwitz	140	<i>A. niger</i> v. Tiegh. mut. <i>echiemanni</i> (Schuem.) n. comb.	224
<i>A. delacroixii</i> (Sacc.) Thom and Church	282	<i>A. niveo glaucus</i> Thom and Raper	135
<i>A. echinulatus</i> (Delacr.) Thom and Church	131	<i>A. nuceus</i> Bloch emend.	202
<i>A. effusus</i> Tiraboschi	267	<i>A. ochraceus</i> Wilhelm	279
<i>A. elegans</i> Gasperini	281	<i>A. oryzae</i> (Vahlburg) Cohn	261
<i>A. fischeri</i> Wehmer	151	<i>A. ostenus</i> Wehmer	263
<i>A. flavipes</i> (Bain. and Sart.) Thom and Church	179	<i>A. panamensis</i> Raper and Thom	217
<i>A. flavus</i> Link	263	1 <i>parasiticus</i> Spence	266
<i>A. foetidus</i> n. sp.	219	<i>A. penicilloides</i> Spegazzini	147
<i>A. fonsccarus</i> n. sp.	227	<i>A. penicillopsis</i> (Hennings) Raeb.	282
<i>A. fumarius</i> Wehmer	237	<i>A. phoeniceus</i> (Cda.) Thom	272
1 <i>fumigatus</i> Frechius	145	<i>A. proliferans</i> G. Smith	117
<i>A. fumigatus</i> (Fres.) mut. <i>helvola</i> Yull	150	1 <i>pseudoglaucus</i> Bloch	110
1 <i>giganteus</i> Wehmer	95	1 <i>pulchellus</i> (Speg.) Thom and Church	279
<i>A. gracilis</i> Bainier	138	1 <i>pulcrulentus</i> (McAlpine) Thom	223
<i>A. granulatus</i> Raper and Thom	175	<i>A. quadrilineatus</i> Thom and Raper	160
<i>A. humicola</i> Chaudhuri and Sachar	193	1 <i>querinus</i> (Bain.) Thom and Church	276
<i>A. itaconicus</i> Hinooshita	142	<i>A. repens</i> (Cda.) DeBary	103
<i>A. janus</i> Raper and Thom	187	<i>A. restrictus</i> G. Smith	141

<i>A. rudor</i> (Brem.)	114	<i>A. terricola</i> Marchal	23
<i>A. rugulosus</i> Thom and Raper	160	<i>A. terricola</i> var <i>americana</i> Marchal	23
<i>A. sclerotiorum</i> Huber	278	<i>A. umbrosus</i> Baimier and Sartory	179
<i>A. sparsus</i> Raper and Thom	253	<i>A. unguis</i> (Fmile Weil and Gaudin)	
<i>A. sulphureus</i> (Fres.) Thom and Church	273	Thom and Raper	169
<i>A. sydowii</i> (Bain and Sart.) Thom and Church	194	<i>A. ustus</i> (Baimier) Thom and Church	171
<i>A. tamaris</i> Kita	254	<i>A. ustus</i> (Bain.) Thom and Ch. var <i>laevis</i> Blochwitz n. comb.	175
<i>A. terreus</i> Thom	195	<i>A. variegator</i> (Berk. and Br.) Thom and Raper	163
<i>A. terreus</i> Thom var <i>aureus</i> n. var	199	<i>A. variegator</i> (Vuill.) Tiraboschi	190
<i>A. terreus</i> Thom var <i>boedijnii</i> n. var	197	<i>A. violacea fuscus</i> Gasparini	231
<i>A. terreus</i> Thom var <i>floccosus</i> Shih	193	<i>A. wentii</i> Wehmer	246

INDLA

Abbott F V 174 1,5 319

Accepted species 360

Air production

Aconitic 299

Amino 299

Aspergillie 299

Citric 23 219 290-293

Fumaric 23 290-294

Gallie 23 291

General 291 295

Glaucic 299

Gluconic 238 295-299

d-Gluconic 299

Glucuronic 299

Glycolic 299

Glyoxylic 299

Itaconic 143 294 296 299

Kojic 210 298 299 299 299

Malic 299

Melleic 299

Oxalic 238 298-299

Aconitic acid 299

Actinomyces 42 31

Agar slant cultures 31

Urbino forms 206 212

Alcohol 316

Alcoholic fermentations 270

Alexander D F ix

Alkaline reaction by *A. clavatus* 92

All rgy 154 271

Alliopsis 9

Alphabetical check list of species and genera 330

Amann J 47 319

Amino acid formation 299

Anastomoses 65

Anslow and Raistrick 154 319

Antitoxics 98

from *A. clavatus* 98

from *A. giganteus* 98

from *A. flavipes* group 182

Names are cited in this index for recognized or historically important species only For a complete list of published names for the *Aspergilli* see the Check List of Species Chapter XXIV

from *A. flavipes* group 299

from *A. fumigatus* 1,4

Arlington Farm 299

Ascogone 299

Ascomycetes 6

Ascothorax nigrans 9

Ascomycota 28

Color in *A. nidulans* group 28 1,6

Germination 2, 29

Markings 108 130 152 162

in *A. fischeri* 162

in *A. glaucus* group 108 130

in *A. nidulans* group 162

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

Aspergillaceae 6

- A. carneus* (van Tiegh) Bloch 201 - 202 199 200
A. carnoys (Biourge) Thom and Raper 134*-135
A. chevaliers (Mangin) Thom and Church 118*-120 104 106 108 119
A. chevaliers (Mangin) var *intermedius* Thom and Raper 121 119
A. citrisporus von Hohnel 251* 51
A. clavatus Desm 92 -95 93 94
A. conicus Blochwitz 140
A. delacroixii (Sacc) Thom and Church 252*
A. echinulatus (Delacr) Thom and Church 131 -137 128 130 106 109
A. effusus Tiraboschi 267 -269 268 69
A. elegans Gasperini 281
A. fischeri Wehmer 151 -153 149 152
A. flavipes (Bainier and Sartory) Thom and Church 179*-181 180
A. flavus Link 263 -266 264 265 69
A. foetidus n sp 219 217 40
A. fonscaeus n sp 227 -228 76 221
A. fumaricus Wehmer 226
A. fumigatus Fresenius 149 -151 149
A. fumigatus (Fres) var *helvola* 63 72 150
A. giganteus Wehmer 90 -97 96 239
A. glaucus Link 3 4 101
A. gracilis Bainier 138 -139
A. granulatus Raper and Thom 175 - 178 176 177
E. herbariorum 4
A. humicola Chaudhuri and Sachar 193
A. staconicus Kinoshita 142 -143 109
A. janus Raper and Thom 45 97 187 - 190 188 46
A. janus var *brevis* Raper and Thom 190
A. japonicus Saito 230
A. luchuensis Inui 230*
A. lutescens (Bain) Thom and Church 251 -253 252
A. mangini (Mangin) n comb 127 -129 128
A. medius Weiss 133 -134 46 128
A. melleus Yukawa 279
A. microcirado citrinus Cost and Lucet 263
A. miyakoensis Nak Sano and Wat 220-221
A. monteridensis Talice and MacKinnon 125* 123
A. nidulans (Eidam) Wint 156*-159 167 168 161 162
A. nidulans mut *alba* Yull 88 77 158 159
A. nidulans var *latus* Thom and Raper 159
A. niger van Tieghem 216 217 218 221, 222 18 72 239
A. niger mut *cinnamomeus* (Schlem) n comb 73 74 206 223 -224 244 247 236
A. niger mut *schlemmii* (Schlem) n comb 73 74 206 224 -225 217 244 242 236
A. niveo glaucus Thom and Raper 133 - 137 18 128 136
A. niveus Bloch emend 202 204 199 200
A. ochraceus Wilhelm 279 -281 280
A. oryzae (Ahlburg) Cohn 261 -263 262 264 69
A. ostianus Wehmer 253
A. panamensis Raper and Thom 21 - 244 243 249
A. parasiticus Speare 266 267 268 70
A. penicilloides Speg 142
A. penicillopsis (Hennings) Rastb 282 - 283
A. phoenicis (Cda) Thom 222 223 218 239
A. proliferans G Smith 117
A. pseudo glaucus Bloch 110 -111 108
A. pulchellus (Speg) Thom and Church 228
A. pulverulentus (Mc Alpine) Thom 223
A. quadrilineatus Thom and Raper 160 161 163
A. quercinus (Bainier) Thom and Church 276*-278 277
A. repens (Cda) De Bary 103 109, 104 105 106 108 109
A. restrictus G Smith 141 136 139 109
A. ruber (Bremer) n comb 114 117 104 106 108 113 115
A. rugulosus Thom and Raper 160 163 158 161 162
A. sclerotiorum Huber 275 279 277
A. sparsus Raper and Thom 283 285 284

- A. salphus* (Fres.) Thom and Church 25 76
A. sydowii (Bain and Sart.) Thom and Church 184 146 185
A. lama is *Kita* 254 257
A. terreus Thom 66 195 196 199 200
A. terreus var. *au-eus-n* var. 66 67 195 196
A. terreus var. *boedijnii* (Bloch) n. comb. 66 67 197
A. terreus var. *floccosus* Shih 66 67 195
A. terricola Marchal 253
A. terricola var. *americana* Marchal 253 254 252
A. umbrinus Bain and Sart. 179 131 123 130
A. unguis (Frut. Weil and Thom and Raper 169 170
A. ustus (Bainier) Thom and Church 171 175 177 173 178
A. ustus var. *laccus* Bloch 175 173
A. variegatus (Berk. and Br.) Thom and Raper 163 166 168 181 182 164
A. versicolor (Lull.) Tiraboschi 190 - 193 185 191
A. violaceo-fuscus Gasperini 231 217 218
A. ventii Wehmer 246 - 247 248 249
 Assumptions Basic 10
- B
- Baarn Holland—See Centraalbureau
Bacillus anthracis 154
 Bacteria in Aspergillus cultures 20
 Bactericidal agents—See Antibiotics
 Bainier G. vii 4 1 4 277 279 236 278
 Bainier and Sartory 107 319 114 129 133
 Barham and Smits 270 298
 Barnes B. 74 144 145
 Barthel C. 56
 Bary A. De vii 4 101 28 28 2 103
 Basidia 23
 Berkeley M. J. 163 165
 Bernhauer K. I. 237 293 295 299 320 291 238 306
 Berntson H. S. 184 320
 van Beyma F. H. viii
- Bibliographies types included viii
 Check list of species with bibliographic references 331 330
 General viii 319-330
 Topical viii 289-318
 Bichloride of Mercury 61
 Binocular wide field 40 45 51
 Biourge J. H. ix 4 133 139 214 232 235
 Bisby G. R. ix
 Black Aspergilli—see *A. niger* group
 Blakenlee A. F. 41 370 229 230
 Blase 22
 Blochwitz Adalbert ix 4 61 66 137 145 197 202 203 206 229 231 236 246 267 279
 Bonner J. T. 242
 Borzi A. 163 163 371
 Brant Nancy iii
 Brefeld O. 4
 Bulb disease 246 249
 Buller A. H. R. 65 371
 Bushan G. Goth 27. 300
- C
- Calam Oxford and Raistrick 204 371
 Candidus group 206-213
 Group relationships 217
 Occurrence 213
 Other white Aspergilli 206 207 212
 Outstanding characters 206
 Sclerotia 212
 Variation in head size 209
 Capitulum 3
 Carbon dioxide ice 53
 Cardozo D. M. 223
 Cathode rays 5
 Cellulose composition 150
 Centraalbureau voor Schimmelfcultures viii 231 24 261 166
 Chaetomium 239
 Challenging retai 270 197
 Characters Diagnostic 82
 Chemistry of Mold Tissue 301 302
 Chitin 317
 Chlamydo pores 28
 Chowdhuri and Mathur 163 321
 Christensen L. M. 271 304
 Chromotaxia Saccardo 242 273
 Church M. B. ix
 Ciferri R. 166 3 1

- Citric acid 237 249 290-293
 from *A. niger* 237 See also 290-293
 from *A. wentii* 249
 Citrinin 205
Cladosporium olivaceum Vuille 9 ■ 73
 240
 Barnes Creamy 74 145
 Conidium formation 73
 Morphology of 73
 Nuclear behavior 73 74
 Clarke F F 198 199
 Classification ■
 Clavacin 98
 Clavatin 99
Clavatus group 92-99
 Antibiotics 93-99
 Occurrence 98
 Outstanding characters 92
 Synonyms 98
 Coffee fermentation 286
 Coghill R D ix 75 376
 Colony characters 11-16
 Colony types 11-13 12
 Color 13
 in conidial walls 14
 in conidiophores 14 ■ 148 153 171
 179 273
 as group characters 13
 in the mycelium 13
 influence of pH 14
 in the substratum 15
 Color photographs of *Aspergilli* Plates
 I-V II
 of *A. alliaceus* Thom and Church Plate
 VI D
 of *A. amstelodami* (Vang) Thom and
 Church Plate III E
 of *A. arenaceus* Smith Plate VI F
 of *A. candidus* Link Plate VI A
 of *A. carneus* (v Tiegh) Blochwitz
 Plate V F
 of *A. clavatus* Desm Plate III A
 of *A. flavipes* (Bain and Sart) Thom
 and Church Plate IV F
 of *A. flavus* Link Plate VII D
 of *A. fumigatus* Fres Plate IV B
 of *A. giganteus* Wehmer Plate III B
 of *A. janus* Raper and Thom Plate I
 E and F Plate V B
 of *A. nidulans* (Edam) Wint Plate
 I A-D Plate IV C
 Color photographs of *Aspergilli*—Cont'd
 of *A. niger* group \ R R L 67 Plate
 VI B
 of *A. niger* group tan spored mutant
 Plate VI C
 of *A. nireo-glauca* Thom and Raper
 Plate III F
 of *A. ochraceus* Wilhelm Plate VII F
 of *A. oryzae* (Vahlb) Cohn Plate VII C
 of *A. quercinus* (Bain) Thom and
 Church Plate VII E
 of *A. repens* (Cda) De Bary Plate III
 C
 of *A. restrictus* Smith Plate IV A
 of *A. ruber* (Bremer) Plate III D
 of *A. sydowii* (Bain and Sart) Thom
 and Church Plate V A
 of *A. tamaris* Kita Plate VII II
 of *A. terricola* var *americana* Marchal
 Plate VII A
 of *A. terreus* Thom (unirradiated)
 Plate II A Plate V F
 of *A. terreus* (L-V mutants) Plate II
 B-F
 of *A. ustus* (Bain) Thom and Church
 Plate IV F
 of *A. variegatus* (Berk and Br) Thom
 and Raper Plate IV D
 of *A. versicolor* (Vuille) Tiraboschi
 Plate V C and D
 of *A. wentii* Wehmer Plate VI F
 Conidia 18 19 24
 Coloration 13
 Endogenous 23
 Formation 23 24
 Germination 29
 Nuclear behavior 23
 Conidiophore or stalk 1, 18 19
 Color 22
 Surface 19
 Connective 24
 Contaminants ■ -67
 Bacteria 59
 Elimination of 41-42 53 59
 Mold disease 59 60
 Other molds 53
 Recognition of 58
 Cook and Lacey 27 301
 Corda A C 1 3 223
 Coremia 13
 Cramer C 4 239 371

- Culture Collections 50
 American Type Culture Collection, Washington 50
 Centraalbureau voor schimmelcultures Baarn Holland 50
 National Type Culture Collection London 50
 Northern Regional Research Laboratory Leonia Ill 50
 Thom Collection 50
 Culture media See Media
 Culture purification of 41 47
 Cultures types of III
 Dilution cultures 40
 Hanging drop 45
 Single colony inoculation 39 40
 Single spore cultures 47
 Spot inoculation 39
 Streak cultures 41
 Three point inoculation 39 40
 Currie J N 237 328
 Curzi M 163 166 371
 Czapek's solution agar III
 D
- Dale Elizabeth 77 101 371
 Dangeard P A 77 37 28 30
 De Bary A See Bary A De
 Descriptive sheet 87
 Descriptive terms 11
 Desiccation of molds vacuum III
 Dewar flask 53 61
 Diastatic enzymes 250 257
 Digestin 213
 Dilution cultures 40
 Dimargaris 9
 Diplocephalus Langeron 8
 Disjunctor 25
 Dodge B O 111
 Dodge C W 5 154 32
 Doelger and Prescott 237
 Dot A W 239 372 32
 Dried cultures—See *Lyophil* preservation of molds
 Dried specimens 67
 Driente 53
 Dual character of 1 *genus* 188 189
 E
- Earle F R 1:8
 Ecads 64
- Edam I 1:5 1:6 327
 Edamsche blazen 28
 Elser W J et al 53 377
Emicella varicolor Berk and Br 9 163
 Immons C W 199
 Endogenous conidia 2
 Engler and Prantl 6
 Enzymes 238 249 713 2 770 771 191 307 308
 Enzyme production
 by 1 *flavus* *ory* *ae* group 770 771 30 304
 by 1 *A niger* group 238 304 305
 by 1 *okala* 713
 by 1 *tamaris* group 2
 by 1 *ventis* group 19
 Ergosterol 191 317
Escherichia coli 154
Fusarium Ludwig 9
 Eurotiaceae 6
 Eurotiales 6
Eurotium Link 7 101
Eurotium herbariorum 101 102 1
- F
- Fat production 194 238 307 50
 Ferdinandson and Winge 17 108 777
 Fermentation Division N R R I 14 20
 Fernbach A 238 304
 Fish fermented 254
 Fisher Ed 165
 Flavocidin 777
 Flavicin 277
Flavipes group 1 3-187
 Antibiosis 187
 Color reactions 190
 Occurrence 182
 Outstanding characters 179
Flavus-ory *ae* group 257-272 69 68
 Antibiosis 271 272
 Enzyme production 270-771
 Kojic acid 270
 Moldy bran 271
 Occurrence 769
 Outstanding characters 259
 Pathogenesis 271
 Variation in 68-69 260
 da Fonseca Olympio 227 230 322

- Foot-cell 17 19
 of *A. effusus* 264
 Frazer and Chambers 27 322
 Fresenius G. vii 4 275 148
 Fumaric acid 237 293-294
 Fumigacin 154
 Fumigatin 154
Fumigatus group 149-154
 Allergy 154
 Antibiosis 154
 Economic importance 153
 Occurrence 153
 Outstanding characters 148
 Pathogenicity 154
 Thermophilic habit 153
 Fungi Imperfecti 6

G

- Gallic acid 238 294
 Galloway L. D. 75 322
 Gene mutation 63
 General bibliography 319-330
 Generic diagnosis 6
 Gilman and Abbott 202 322
 Glauic acid 299
Glaucus group 100-147
 Economic importance 148
 Group relationships 102
 Historical considerations 101
 Laboratory cultivation 101
 Occurrence 146
 Outstanding characters 100
 Pathogenicity 146
 Pigment formation 15
 Temperature relations of 45 133 134
 Variation in 143-145
 Glister G. A. 272 300
 Gluconic acid 238 295-297
 d-Gluconic acid 299
 Glucuronic acid 299
 Glycolic acid 299
 Glyoxylic acid 299
 Gould H. S. 258 295
 Gould and Raistrick 15 323
 Graphic key to groups 87
 Greene H. C. 65 323
 Greene and Fred 56 323
 Group keys
A. candidus group 207
A. clavatus group 92

- A. flavipes* group 179
A. flavus-oryzae group 209-260
A. fumigatus group 148
A. glaucus group 102
A. nidulans group 155-156
A. niger group 215-216
A. niger group (Mos. cray s.) 232 235
A. ochraceus group 274-275
A. tamarii group 250-251
A. terreus group 195
A. ustus group 171
A. versicolor group 183
A. wentii group 241
 Growth substances 316
 Guegen F. 4 323
 Gum 317

H

- Haines R. W. ix Plates I-V II
 Haller D. A. 3
 Hanging drop culture 45
 Hansen H. N. ■ 323
 Hansen and Smith 65 323
 Hanzawa J. ix 225
 Hao L. C. 271 303 304
 Hay infusion agar 35
 Head 16 18-21
 Henrard P. 27, 323
 Herbarium specimens 69
 Herrick H. T. ix 237 238 277
 Heterothallism 27
 High sugar Czapek's agar 101
 Historical introduction 3
 Hollaender A. 75 326
 Homothallism 27
 Hooper et al. 99 323
 Huber ■ A. 278 286 323
 Hülle cells 23
 in *A. carneus* 176 202
 in *A. flavipes* group 179
 in *A. nidulans* group 157 167
 in *A. ustus* group 176
 in *A. versicolor* group 188 197
 Hydrogen ion concentration 14 ■ 35
 H₂O
 Hydroxylamine 317
 Hyphomycetes ■

I

- Identification of *Aspergilli* 81-91
 Incubation of stock cultures 51

INDEX

- Incubators 49
- Induced variation 4
- Industrial Farm Products Research Division 22
- Inoculating needles and loops 47
- Intermediate species 86
- Interpretation of Descriptions 10
- Inui T 230
- Invertase deficiency in *A. panamensis* 711
- In. erythrospora* Borsari 9
- Isolation of single spores 43 44
- Itaconic acid 304 305 143 27
- J**
- Jardin Botanique de l'Etat 235
- Jones Hake and Hamre 301
- K**
- Karrow E O 719 723 323
- Katsunobushi 246
- Keith, G W 43 323
- Keys 86-91
 - based on color 88-89
 - based on morphology 90-91
 - Graphic 87
 - to groups 86-91
 - to species—see group keys
- Kinoshita K 147 143 323 324
- Kita G ix 253 300 321
- Kluyver and Perquin 270 298
- Koji 38 719 237 260
- Kojic acid 49 238 270 297 298
- L**
- Lacto-phenol 49
- Lambert E B 43 374
- Langeron M 204 324
- La Rue C D 43 324
- Ledingham G A ix 198
- Link H F 3 101 210
- Linossier G 208 374 15 236
- Lockwood et al 76 324 305 297
- Loops transfer 47
- Ludwig F 8 374
- Lutz L 226
- Lyophil preservation of bacteria 53
- Lyophil preservation of molds 53
- Advantages of 56
- Apparatus 54
- Methods 53 55
- Recultivation 53
- Viability 51
- M**
- Ma Roberts ix
- Macrosporgilli 16
- Macy H ix
- Maintenance of cultures of 3
- Malic acid 299
- Malt extract agar 33
- Mangin M I 119 177 178 143
- Mannitol 317
- Manual use of 81 91
- Marchal F 233 321
- Martin C W 6
- Mary Clare Sister 265
- May O F ix 227 237 238 270
- McCoy Prof Elizabeth 36
- McKee and MacPhillamy 272 301
- McKee Hake and Houck 272 301
- Mealy bugs 707 260
- Media culture 31
- Czapek solution agar 32
- Hay infusion agar 33
- High-sugar Czapek agar 101
- Influence of 36
- Malt extract agar 33
- Mosses and Maulin's solution 34
- Neutral Raulin's solution 33
- Sporulation 37 38
- Steep liquor Czapek's agar 33
- Steinberg's solution 34
- Melleic acid 799
- Metarrhizium 239
- Methyl cellosolve 53
- Micheli P A vii 3 100
- Microsporgilli 16
- Microbiological Congress Third Inter national 5
- Microscopes 48
- Mildew 146 239
- Mites 60
- Damage caused by 61
- Elimination of 61-62
- Poison 61
- Mixed cultures 57
- Mold Disease of *A. nige* 59 60
- Mold Tissue Composition of 301 302

- Moldy bran 271
 Mollard M 238 293
Monilia 3
 Monograph 5
 Monospore Cultures 42
 'Monster' 74 85
 Montagne J F 3
 Morphology and Description 10
 Morrow Marie B ix
 Mosseray Raoul ix 70 75 88 229 232 235
 Mosseray's Synopsis of Species 232 234
 Mounting fluid 47
 Moyer A J 37 227 237 238 258 270
 Moyer and Coghill 205 297
 M type and C type 65
 Mucidinaceae 6
 Mucidineae 6
 Mucoraceae 42
Mucor herbariorum 7
 Mutant definition of 63
 Mutation 63
 Mutations deficiency 76
 Mutations induced 74
 by cathode rays 75
 by chemicals 74 75
 by heat stimulation 74
 by ultra violet radiation 75 76 77
 in *A. glaucus* group 74
 in *A. niger* 75
 in *A. terreus* 75
 Mutations injury 75
 Mutations morphological 18 77
 Mutation natural 71
 in *A. fumigatus* 72
 in *A. glaucus* group 73
 in *A. nidulans* 72
 in *A. niger* 72 73
 Mutations physiological 78
 Mutation taxonomic usage 11
 Nakazawa R ix 225
 Nakazawa Simeo and Watanabe 220
- N
- Natural groups 82 87
 Natural Mutation 71
 Natural relationship of groups 87
 Needles transfer 47
 Neill J C 5 69 325 83 143 193
 Neutral Raulin's solution 33
- New species 84
 Bases for description 85
 Recognition of 84
Nidulans group 153-154
 Occurrence 170
 Outstanding characters 153-156
 Pathogenicity 170
Nig r group 214 240
 Citric Acid 237 290-293
 Coloration 236
 Conidiophore structure 19 222
 Enzyme production 238 304 305
 Fat production 238 306
 Fumaric Acid 237 293-294
 Gallic Acid 237 294
 Gluconic Acid 238 295-299
 Industrial strains preservation 240 50-62
 Mildew 239
 Mosseray's Synopsis of Species 232-234
 Occurrence 236
 Outstanding characters 214
 Oxalic acid 238 295-299
 Pathogenesis 239 240 30 310
 Physiology 239 310-317
 Soil analysis 238 313-314
 Strain 67 277
 Variation in head structure 218
 Variation in spore size 221
 Niklas H 233 313
 Northern Regional Research Laboratory vii 50
- O
- Ochraceus* group 3 296
 Fermentations 296
 Occurrence 295-296
 Outstanding characters 293
Ochraceus 319
 Odor
 Actinomyces like in *A. foetidus* 210
 foetid in *A. claratus* 20
 foetid in *A. flavipes* 180
 Okazaki K 213 211 325
Oryae group—See *Flarus* group
 Oshima K ix 260 303 261 270
 Ota M 271 308
 Overgrowths 58
 Oxalic acid 238 295-299
 Oxford and Rastick 154 325

I

- Parasiticin 77
 Parasitism of *Aspergilli* 59
 Partaneky and McIlherson 239 32
 Pathogenicity 307 310
 in *A. flavus-oryzae* group 271
 in *A. fumigatus* group 151
 in *A. glaucus* group 146-147
 in *A. nidulans* group 109-170
 in *A. niger* group 239-240
 in *A. terreus* group 204
 in *A. versicolor* group 193 194
 Patouillard and Delacroix 222 223
 Patulin 111
 Penicillin 99 142
 Penicillin like substances
 in *A. farinosus* 182
 in *A. flavus* 72
 in *A. giganteus* 66
Penicillium chrysogenum Thom 57
Penicillium notatum Westling 272
Penicillium patulum West 99
Penicillium restrictum Abbott 67 146
Penicillium rugulosum Thom 59
Penicillium spinulosum Thom 151 300
 Penicillium 26
 in *A. fischeri* 149 151 153
 in *A. glaucus* group 27 100-137 106
 115
 in *A. nidulans* group 155-166 161 164
 Person C H 3
 Pfizer (Chas.) and Co Inc ix Pls I VII
 Phialids 111
 Philpot F J 99 301
 Photographic equipment 49
 Physiology 239 310-312
 Pigments 15 143 236 312 313
 Plectascineae 6
 Plug poison 61
 Poison cotton plugs 61
 Polysaccharides 318
 Pontillon C 238 306
 Preservation of Cultures 111
 in agar slants 111 52
 in lyophil form 53-56 111
 in soil 56-57 52
 on vegetable substrata 57
 Industrial strains 240 50-62
 Progressive variation 68

Isoolytic enzyme 213 257 71
 Jullis 214

Q

Quilico and In Capua 15 313

R

- Raistrick Harold ix 111 117 113 231
 238 243
 Raistrick Robin on and Todd 15 313
 Rams 23
 Raper Coghill and Hellander 15 376
 205 206 717 22
 Raper and Thom 106 175 187 190 717
 253
 Raulin J 4 237
 Raulin neutre gelatin 70 111
 Raulin's solution 34
 Recultivation of dried cultures 55
 Reduced structures in *A. sydowii* 184
 Rejected species—See Check list of
 species
 Resting nuclei 73
 Ridgway Robert 13 376
 Ridley H V 13 64 376

S

- Saccardo L A 11 160 376
 Saccharification 71
 Saito K 231 77
Salmonella typhi murinum 184
 Saltant 64
 Sartory A 4
 Sartory and Meyer 217 327
Sarotya fumigata 9 153
Scales F M 243
 Schumann Elizabeth 74 723 224 327
 206
 Schmidt C F Jr 238 306
 Schwartz W 30 377
 Sclerotia 30 712
 in *A. candidus* group 212 208
 in *A. flavus-oryzae* group 259 265
 in *A. niger* group 714 217
 in *A. ochraceus* group 773 277
 in *A. tamarii* group 250
 in *A. versicolor* group 211 243
 significance of 212
 structure of 30
 Secondary growth 58

- Sectors 61
 Septa 17
 Shih Y H ix 198
 Short lived species 51
 Siebenmann F 271 307
 Simonart Paul ix 232
 Single spore cultures 42 44
 Smear cultures 41
 Smith George ix 5 117, 102 137 139
 141 246 285
 Sodium lauryl sulfonate 37
 Soil analysis 238 313-314
 Soil Fungi Summary of 202
 Soil Preservation of Molds 56
 Directions for 56
 Viability 56
 Sopp O J O 9
 Soy products including soya sauce 249
 258 270
 Speare A T 260 266 328
 Species
 Accepted 360-361
 Check list of viii 331-359
 Definition of 83
 Diagnosis of 82
 Keys to see group keys
 New 84
 Rejected—See Check list of species
 Transitional 86
 Specimens identification from 81
 Spegazzini C 8 279 328
 Spinulosis 154
 Spores
 Ascospores 28 29 162 162 130 108
 Conidia 23-25 54 221
 Sporodinia 3
 Sporulation media 37 38
 Sprays 62
 Stalk—See Conidiophore 17
 Staphylococcus 51 98 99
 Staphylococcus aureus 154 182
 Steep liquor Czapek & agar 35
 Steinberg R A 239 310 311 312
 Steinberg & nutrient solution 34
 Steinberg and Thom 75 328 206 212
 224 225 240
 Stigmata 22
 Primary 19 23
 Secondary 19 23
 Sterigmatocystis Cramer 8
 Sterile hyphae in *A. unguis* 169
 Stock cultures 51 57
 Agar slants 51
 Cultivation 51
 Incubation 51
 Periodic transfer 51
 Storage 51
 Streak cultures 41
 Streptococcus viridans 154
 Substrata see Media
 Swift Marjorie E 111
 Synonyms
 in *A. candidus* group 211
 in *A. chevalieri* series 120 121
 in *A. clavatus* group 98
 in *A. echinulatus* 133
 in *A. flauus oryzae* group 760 769
 in *A. luchuensis* series 232
 in *A. niger* series 225-276
 in *A. nireo glaucus* 137
 in *A. ochraceus* series 281
 in *A. repens* series 107 110
 in *A. restrictus* series 139
 in *A. ruber* series 117
 in *A. sulphureus* series 2 6
 in *A. sydowii* series 166
 in *A. tamaris* group 756-767
 in *A. terreus* group 197 200
 in *A. umbrosus* series 131
 in *A. versicolor* series 197 198
 in *A. wentii* group 21 718
 synonymy 6
 System und Phylogenie 5
 T
 Takahashi T 200 328
 Takamine J 7 0 307
 Tamaris 258
 Tamaris group 250-258
 Economic importance 257 258
 Occurrence 257
 Outstanding characters 250
 Tamura and Morita 4
 Tannic acid 237 294
 Tannin 237 294
 Taubenhaus J J ix
 Temperature 43 46
 Temperature effect of
 on *A. giganteus* 45 97
 in *A. glaucus* group 45
 on *A. janus* 45 187
 on *A. medius* 45 46 131

- Temperatures optimum 45
 Terminology descriptive 11
 Terrain 318
Terreus group 193-203
 Antibiotics 203
 Itaconic acid 204 205 207
 Mutations 25
 Occurrence 204
 Outstanding characters 195
 Pathogenesis 204
 Variation in 66 67 197
 Tessar lenses 49
 Thaxter R T 10 159 251
 Thermophilic species 45
 Thom C vii 5 195 323
 Thom and Church vii 4 5 107 137 206
 235 255 260
 Thom and Raper 4 323 107 168
 Thom and Steinberg 75 323 141 150
 21 221
 van Tieghem Ph vii 4 237
 Timonin M I 203 205 329 213
 Topical bibliography 287-318
 Transfer needles and loops 47
 Transfer of stock cultures 51
 Transitional species 80
Trichoderma 47
 Turfitt G E 246
 Type culture collections 50
- U
- Ultra violet radiation 75 76 77
 Underkoffler et al 271 303 285
Utilago phoenicis Cda 223
Ustilago group 171 178
 Hulle cells types 178
 Occurrence 178
 Outstanding characters 171
- V
- Vacuum desiccation of molds 53
 Vacuum tester 55
 Variant 64
 Variation natural 63-78
 in *A. fischeri* 65
 in *A. flavus* *oryzae* group 63 69
 in *A. fumigatus* 68
 in *A. niger* group 60 71
 in *A. sydowii* 67
 in *A. terreus* group 66 67
 Intra group 68
 Intra species 68 67
 Intra strain 65
 Variation references 314-315
 Variety taxonomic usage 64
 Vegetable substrata 57
Versicolor group 183 194
 Occurrence 194
 Outstanding characters 183
 Pathogenesis 193
 Strain variation 19 186
 Vesicle 18 19 22
 Vestigial characters 87
Vibrio cholerae 154
 Vitamin D 238
 Vitamins 316
 Vuillemin P 163 379
- W
- Waksman and Bugie 277 301
 Waksman et al 98 300 99 301 154
 Ward G E 237 270 379
 Webb P H W 97 379
 Wehmer C vii 4 226 237 238 247 253
 Weisner B P 98 300
 Wells May Moyer Herrick et al 227
 231 270
Wentia group 241 249
 Economic importance 249
 Occurrence 249
 Outstanding characters 241
 Westerdijk Johanna viii 110 142 283
 Whelden R M 75 330 206 225
 White F C 182 330 271 300 272
 White mutants 206
 Wickerham L J 35
 Wickerham and Andreasen 53 330
 Wiggers Fredericus Henricus 100
 Wilhelm H A vii 4 231 330
 Wilkins and Harris 99 300
 Wire nichrome 47
 platinum iridium 47
 Wolf F A 97 330
- Y
- Yabuta T 249 297 330
 Yull Edward 63 150 159 206 212 221
 Yull John and Edward ix 73 145 240
 Yukawa M 279 286 330
- Z
- Zea Mays 267
 Zonation 11-13 12

